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Dames and Moore, Los Angeles, CA

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This project consisted of two simultaneous phases. One phase was the construction and preliminary application of a prototype, experimental wastewater treatment seawater aquaria research facility. This facility, the Sewage Treatment and Bioeffects Laboratory (STABEL), was designed to replicate, as closely as possible the wastewater treatment process, the outfall disposal method, and the surrounding marine environment found along the southeast cost of Florida. The other phase was the collection of field data for the establishment of operating parameters, comparison data and baseline information, so that a context for the interpretation of experimental results could be developed. This included the collection of operating parameters and data from the largest sewage treatment plant discharging wastewater to the study area. Data also were collected to evaluate the *in situ* impact of such wastewater discharges upon corals, selected sessile invertebrates, and finfish, and to provide baseline information on the fates of the bacterial and viral fractions of untreated and treated wastewater discharges off southeast Florida.

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PREFACE

The Southeast Florida Ocean Outfall Study (SEFOOS) project was designed to provide the U.S. Environmental Protection Agency (EPA) with scientific information relating to marine ecological effects associated with the discharge of treated municipal wastewaters through ocean outfalls along the southeast Florida coast. The information, obtained between 1976 and 1981 under EPA Grants No. R-804749 and R-806307 to the Rosenstiel School of Marine and Atmospheric Science of the University of Miami, was sought by EPA to carry out the provisions of PL 82-500, the Federal Water Pollution Control Act Amendments of 1972. Specifically, this information was to be used in establishing (or revising) effluent quality criteria under Sections 301 and 403, water quality criteria applicable to marine waters (Section 303), and best practicable treatment to be employed by 1983 (Sections 201 and 301) for municipal wastes discharged via ocean outfalls to waters of the territorial sea, the contiguous zone, or the open ocean.

Owing to a reduction in EPA funding for research, the SEFOOS project was terminated before completion of the planned studies. Therefore, under EPA Contract No. 68-03-1706, Dames & Moore has collected all available field and laboratory records at the Rosenstiel School of Marine and Atmospheric Science of the University of Miami, in order to analyze, synthesize, and interpret the data and results of the SEFOOS project.

This report presents these data, the methods (if recorded) used to obtain them, and, where available, observations made by the grant's Principal Investigators. Listed below are the names of the component studies of the SEFOOS project, and the name of the corresponding Principal Investigator(s) who had major responsibility for planning of the study, generation of the associated data, and/or summarizations included in interim reports to EPA.

Overall Coodinations and Review - Dr. Samuel C. Snedaker

STABEL Concept and Construction - Dr. Samuel C. Snedaker
Dr. Lanny Udey
Dr. Shaukat Farouq
Mr. George Chipouras

STABEL Operation - Mr. Jack Baird

Virus and Bacteria Studies - Dr. George E. Schaiberger

Coral Reef Field and Preliminary
Laboratory Studies - Dr. Dennis Taylor

Coastal Fishes Field and
Preliminary Laboratory Studies - Dr. Lanny Udey

Wastewater and Tissue Analysis - Dr. Eugene Corcoran

STABEL Experiments with Fish and Shrimp - Dr. Lanny Udey

STABEL Experiments with Corals - Dr. Donald Marszalek

In view of the distinct nature of these components of the multi-disciplinary SEFOOS project, this report has been structured so that each major component is covered in a separate section. Based on the data and observations of the Principal Investigators which have been incorporated into these individual sections, Dames & Moore has analyzed, interpreted, and/or summarized these results, and provided conclusions from the SEFOOS project and recommendations for future research.

ABSTRACT

This research program (the Southeast Florida Ocean Outfall Study - SEFOOS) was initiated to provide EPA with information on the ecological effects of municipal wastewaters discharged to the coastal marine waters of southeast Florida following various degrees of treatment. The program consisted of both field and laboratory studies.

The field phase, conducted near major southeast Florida submarine outfalls and at control sites, included surveys of enteroviruses and indicator bacteria in seawater and sediments; seawater nutrients and associated rates of primary production, calcification, growth in reef-forming corals and gorgonions; and the distribution and health of coastal fish species, including the occurrence of external diseases and abnormal histopathological conditions.

The laboratory phase included preliminary toxicity tests with candidate bioassay species of local fishes, involving static exposures of seawater diluted wastewaters. A computerized literature search of bioassay procedures, and effects of exposure to sewage and major classes of toxicants, yielded more than 400 relevant citations which were tabulated (partially annotated).

A major part of the program was the construction and testing of the Sewage Treatment and Bioeffects Laboratory (STABEL), and development of a successful bioeffects protocol. Raw sewage was treated by (1) primary settling, (2) chemical flocculation, and (3) activated sludge (biological secondary), with and without chlorination, to produce six types of wastewaters which were tested on local fish, shrimp, and coral species at 30:1, 100:1, and 300:1 dilution. Levels of general constitutents, micronutrients, toxic metals, and synthetic organics in these wastewaters, and metals and synthetics in some exposed organisms, also were determined.

The SEFOOS study was conducted by the Rosenstiel School of Marine and Atmospheric Science of the University of Miami between 1976 and 1981 under EPA Research Grants No. R-804749 and R-806307. This final report was prepared by Dames & Moore under Contract No. 68-03-1706.

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Appendix E lists the Principal Investigators and their colleagues who participated in the multi-disciplinary SEFOOS study (under EPA Grants No. R-804749 and R-806307) between 1976 and 1981. This report (submitted in fulfillment of EPA Contract No. 68-03-1706) was prepared in collaboration with Dr. Samuel Snedaker, Mr. Melvin Brown, Dr. Eugene Corcoran, Dr. Lanny Udey, Dr. Don Marszalek, and Dr. George Schaiberger (University of Miami), Dr. Dennis Taylor (University of Maryland), Metro-Dade Water and Sewer Authority, and the Project Officer, Dr. Donald Baumgartner (U.S. Environmental Protection Agency). The full cooperation of these scientists and the administration of the University of Miami in the preparation of this report is sincerely appreciated.

SECTION 1

INTRODUCTION

This project consisted of two simultaneous phases. One phase was the construction and preliminary application of a prototype, experimental wastewater treatment - seawater aquaria research facility. This facility, the Sewage Treatment and Bioeffects Laboratory (STABEL), was designed to replicate, as closely as possible, the wastewater treatment process, the outfall disposal method, and the surrounding marine environment found along the southeast coast of Florida. The other phase was the collection of field data for the establishment of operating parameters, comparison data and baseline information, so that a context for the interpretation of experimental results could be developed. This included the collection of operating parameters and data from the largest sewage treatment plant discharging wastewater to the study area. Data also were collected to evaluate the *in situ* impact of such wastewater discharges upon corals, selected sessile invertebrates, and finfish, and to provide baseline information on the fates of the bacterial and viral fractions of untreated and treated wastewater discharges off southeast Florida.

SECTION 2

CONCLUSIONS

1. Enteroviruses have been measured in sediments more than 3.5 km from a raw sewage coastal submarine outfall in the absence of detectable viruses or indicator bacteria in the overlying seawater. However, further studies are required to determine the source.

2. Minor (2- to 4-fold) elevations of micronutrients (ammonia, nitrate, and orthophosphate) occur in near-bottom seawater around the Virginia Key, Miami Beach, and Broward submarine outfalls. Major gradients in effects on corals or gorgonians in the vicinity of the outfalls are not apparent.

3. Coastal fishes collected off southeast Florida near the Virginia Key discharge of chlorinated (biological) secondary effluent exhibit insignificant external or internal disease symptoms, limited histopathological effects and no abnormal parasitic invasion or hyperplasma of gill tissue.

4. A successful protocol has been developed for field sampling, holding, and feeding a variety of coastal or estuarine marine fishes and invertebrates, and utilizing them in the bioeffects tests at the SEFOOS-STABEL facility.

5. The SEFOOS-STABEL facility can provide wastewater effluents that are representative of those generally produced by major treatment plants, and a nearly-infinite degree of seawater dilution can be obtained. The biggest problem experienced with the facility to date is the difficulty of keeping the residual chlorine level within tolerances (apparently caused by large variations in the chlorine demand of municipal wastewater). The high mortality observed in some Experiment III tests illustrates the importance of chlorination as a source of toxicity in wastewaters.

6. Relatively low concentrations (1-100 ug/liter) of trace metals occur in the STABEL raw and primary treated sewage. Levels of chlorinated pesticides and PCBs range up to a few ug/liter, and are within the range typically reported for municipal wastewaters. However, the concentration of phthalic acid esters ranged up to about 200 ug/liter. Increased treatment by chemical flocculation or activated sludge (biological secondary)

reduces the levels of the five target metals, by an order-of-magnitude in the case of copper and zinc. However, these treatments do not produce observable decreases in the effluent concentrations of the synthetic organics.

7. Based on the STABEL Experiments I and II, the juvenile silver jenny (Eucinostomos gula) is a very hardy bioeffects test organism (no control specimens died after 42 days, and a ten-day exposure to a 30:1 dilution of non-chlorinated primary effluent caused 0 percent median mortality). In contrast, the adult hardhead silversides (Atherinomorus stipes) appears to be overly sensitive (a median mortality of 69 percent for the controls was observed). Surprisingly, survival of the silversides increased systematically as both the level of wastewater treatment and degree of dilution decreased (only 19 percent median mortality occurred for the non-chlorinated primary effluent, diluted 30:1).

8. The adult sheepshead minnow (Cyprinodon variegatus) tested in STABEL Experiment III also is a hardy bioeffects test species. Over a 34-day exposure period, median mortalities of 0, 10, 5, and 1 percent were observed in 30:1 dilutions of non-chlorinated primary, chemical flocculation, and biological secondary effluents, and the controls, respectively. However, apparent over-chlorination (residual values unknown) in the 30:1 dilutions of primary, chemical flocculation, and biological secondary effluents caused respective median mortalities of 100, 85, and 25 percent. For the remaining diluted chlorinated effluents, median mortality values ranged from 0 to 15 percent. Generally, there appeared to be a trend of decreased mortality with increased treatment and dilution. Trace metals were not bioaccumulated substantially above normal. Higher whole-body concentrations of PCB 1254 (0.88 to 1.5 mg/kg dry wt.) were measured in the exposed specimens compared to the control value (0.71 mg/kg), but there was no apparent relationship between concentration and degree of treatment.

9. The pink shrimp (Panaeus duorarum) appears to be an appropriate bioeffects test species. In STABEL Experiment IV, the median mortality for the control specimens was only 5 percent, while a value of 100 percent was obtained after a 35-day exposure to a 30:1 dilution of the non-chlorinated primary effluent. However, for the remaining non-chlorinated tests, these values ranged between 0 and 10 percent, suggesting that the causative agent was at a critical concentration in the 30:1 dilution of the non-chlorinated primary effluent. At a 30:1 dilution, chlorination of the effluents from all three treatments caused significant mortality (median values of 25 to 80 percent); the values were only 5 to 10 percent for the remaining chlorination tests. Thus, increasing dilution from 30:1 to 100:1 reduced considerably the mortality figures observed in the chlorinated effluent tests conducted at the lowest dilution. No abnormal bioaccumulation of target trace metals and chlorinated hydrocarbons in the exposed specimens were observed.

10. Juvenile high hats (Equetus acuminatus) were exposed for a relatively brief (10-day) period to various seawater: effluent mixtures in STABEL Experiment V. At the end of the 54-day observation period, there was substantially higher mortality with chlorination than without at the lowest (30:1) dilution. Further, in the case of chlorination, mortality generally decreased with increasing dilution. For effluents of all three treatments at the lowest dilution (30:1), and for the intermediate dilution (100:1) of primary effluent, median times to death were substantially shorter with chlorination than without. Increasing the dilution from 30:1 to 100:1 greatly increased the survival time of the specimens exposed to the chlorinated effluent from all three treatments.

11. Exposure of coral species, unaccompanied by algal grazers, to diluted wastewaters in laboratory bioeffects tests can result in excessive fouling from luxurient growths of algae stimulated by the wastewater nutrients. Results of the STABEL coral experiment indicate that the level of effluent treatment is the most important factor influencing fouling of the aquaria and potential overgrowth of the coral by algae. Primary treatment resulted in accelerated fouling, regardless of chlorination or dilution. Chlorination also is important in the control of fouling; regardless of dilution, all chlorinated effluents from the chemical flocculation or biological secondary treatments resulted in reduced fouling. Dilution was important in determining the level of fouling in non-chlorinated effluents, especially those treated by chemical flocculation and biological secondary methods.

The concentrations of trace constituents of common concern (e.g., ammonia, trace metals and chlorinated hydrocarbons) measured in the STABEL wastewaters are not toxic to the reef-building corals tested (Monastrea cavernosa and Dichocenia stokesii). In addition, the episodes of over-chlorination during the experimental period, which, in the concurrent Experiment III, caused the high mortalities of sheepshead minnows in the 30:1 dilutions of effluents from all three treatments, caused no measurable mortality of the corals. Thus, chlorination of municipal wastewater effluents discharged near coral reefs may benefit the corals themselves by inhibiting fouling by reef algae. However, the possible toxicity of chlorinated wastewater to a wide variety of marine organisms very probably would out-weigh any benefit derived by the corals themselves in an evaluation of the overall ecological effect of such discharges to coastal marine ecosystems.

SECTION 3

RECOMMENDATIONS

1. The transport mechanisms and distributions in seawater, sediment and biota of both enteroviruses and "indicators" of mammalian fecal material (e.g., coliform bacteria) require further study around marine discharges of municipal wastewaters. Emphasis should be placed on the public health and ecological significance of viruses in nearshore sediments.

2. The suspected unnatural algae overgrowth of corals in the STABLE tests suggests the need for additional field studies on reefs near municipal wastewater outfalls, and the use of more representative communities (e.g., including grazers) in future laboratory studies.

3. The absence of detrimental effects observed in fishes collected around the southeast Florida ocean outfalls suggests that additional surveys of this kind in the region are not needed. However, results from in-depth studies incorporating state-of-the-art histopathological and biochemical techniques might be justified if the anticipated results are intended to test specific hypotheses about the ecological effects of marine discharges off southeast Florida.

4. The variance observed in the results of the preliminary STABEL experiments should be utilized in the design of future bioeffects tests regarding the replication needed to detect specific differences at various levels of confidence.

5. Long-term studies (>100 days) using STABEL facilities are needed to better document chronic effects of exposure to treated wastewater. A wide spectrum of indices of sub-lethal effects should be incorporated into these studies.

6. The problems encountered during these initial STABEL experiments with documented or suspected overchlorination illustrate the critical importance of controlling and monitoring residual chlorine levels in the diluted effluents. Future STABEL programs should focus on chlorination as one of the major contributors to the toxicity of wastewaters.

7. The apparent insensitivity of corals to chlorination should be studied further to establish the tolerance limits of reef-building species.

8. Future bioaccumulation studies should emphasize trace organics over toxic metals, unless the latter studies include the determination of metals in biochemical "pools" of detoxification (e.g., metallothionein-like proteins) and toxic action (e.g., enzymes).

9. The STABEL approach to determine the toxicity of wastewaters as a function of treatment (including chlorination) and dilution should be employed in various regions of the country where major expenditures are being considered to upgrade wastewater treatment for the protection of aquatic ecosystems.

SECTION 4

SEWAGE TREATMENT AND BIOEFFECTS LABORATORY

INTRODUCTION

The largest single component of the SEFOOS project was the construction and initial application of the Sewage Treatment and Bioeffects Laboratory (STABEL). STABEL was designed to give the researcher the capability of investigating the effect of different treatment processes, different chlorination concentrations, and varying dilutions of the treated wastewater with seawater on selected marine organisms. The laboratory offers the investigator five (5) different wastewater types, control over their treatment processes, large selection of dilutions with seawater, enough aquaria for statistically sound research, and large areas for maintaining and acclimating test organisms.

The laboratory is situated on Virginia Key in the City of Miami, Dade County, Florida (Appendix F), on the University of Miami Rosenstiel School of Marine and Atmosphere Science (RSMAS) East Campus. The East Campus is situated in a scientific complex consisting of two U.S. Department of Commerce laboratories and is only 300 meters from the RSMAS Main Campus. This location allows the laboratory to draw on a great deal of scientific expertise from both federal and private institutions.

The actual construction of STABEL began on March 9, 1979 after many months of feasibility studies, planning, designing, acquiring land and permits. The University of Miami accepted ownership of the completed facility on September 30, 1979. It became operational in December 1979, and was used to conduct the bioeffects experiments between December 20, 1979 and August 3, 1980. The construction costs for the completed facility totaled $533,235.00. The costs were shared by the U.S. Environmental Protection Agency and the University of Miami.

DESCRIPTION

Figure 1 is a flow diagram showing the operating capabilities of STABEL. A list of the final engineering drawings for the facility is presented in Appendix A, and labeled photographs illustrating key components of STABEL are presented in Figures 2 through 10. The research facility is designed as a closed system. The laboratory (Figure 2) is supplied with raw wastewater from the Virginia Key Sewage Treatment Plant (STP), which is the largest treatment facility using ocean outfall disposal in Florida. On the average the Virginia Key sewage treatment

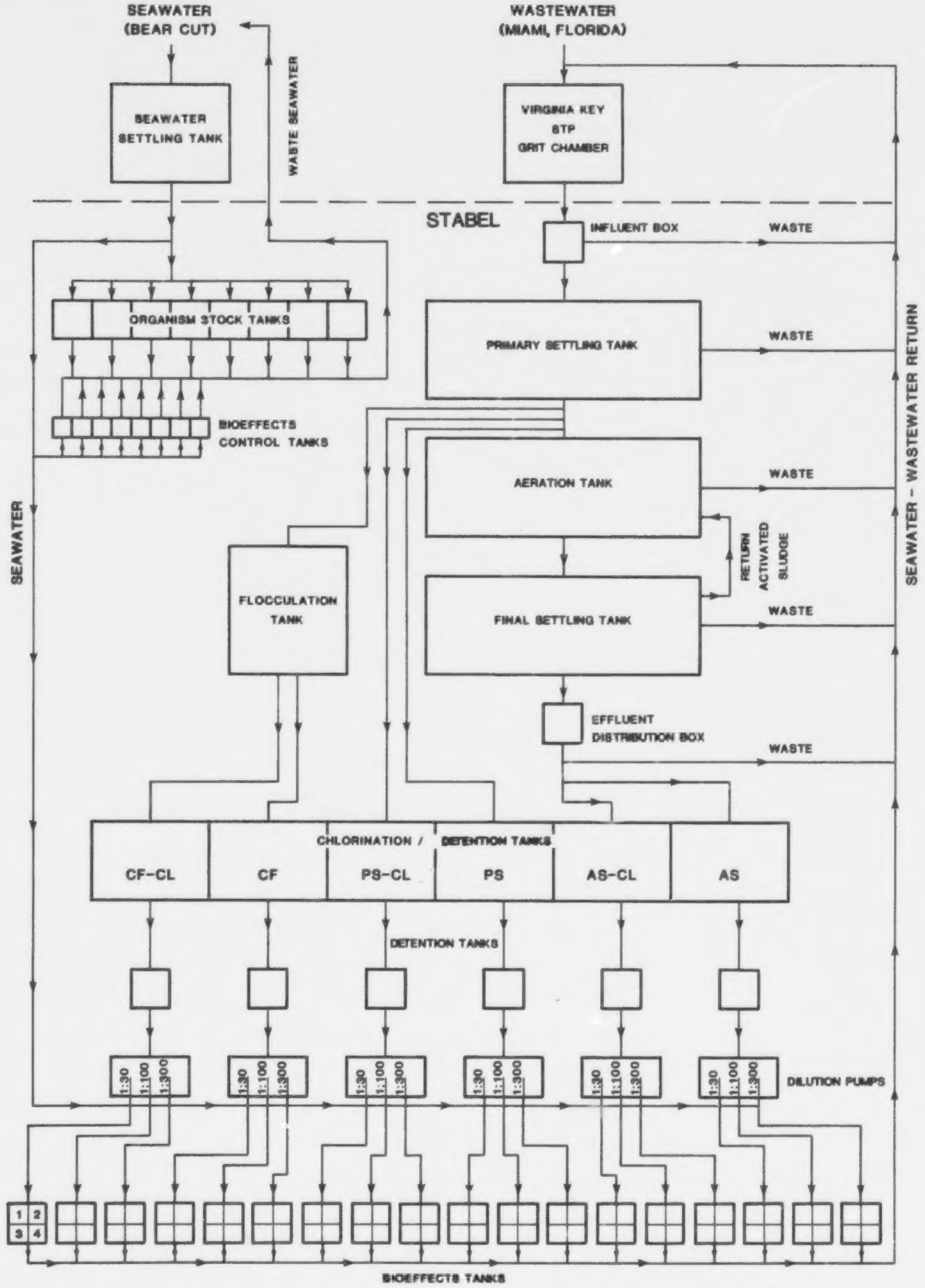
SEAWATER
(BEAR CUT)
WASTEWATER
(MIAMI, FLORIDA)
SEAWATER SETTLING TANK
WASTE SEAWATER
VIRGINIA KEY
STP
GRIT CHAMBER
STABEL
INFLUENT BOX
WASTE
ORGANISM STOCK TANKS
PRIMARY SETTLING TANK
WASTE
BIOEFFECTS
CONTROL TANKS
AERATION TANK
WASTE
SEAWATER
RETURN
ACTIVATED
SLUDGE
SEAWATER - WASTEWATER RETURN
FLOCCULATION
TANK
FINAL SETTLING TANK
WASTE
EFFLUENT
DISTRIBUTION BOX
WASTE
CHLORINATION / DETENTION TANKS
CF-CL
CF
PS-CL
PS
AS-CL
AS
DETENTION TANKS
1:30
1:100
1:300
DILUTION PUMPS
1
2
3
4
BIOEFFECTS TANKS

Figure 1. Flow diagram of the Sewage Treatment and Bioeffects Laboratory (STABEL). The types of treatment available are primary settling (PS), chemical flocculation (CF), and activated sludge biological secondary settling (AS), without or with chlorination (CL).

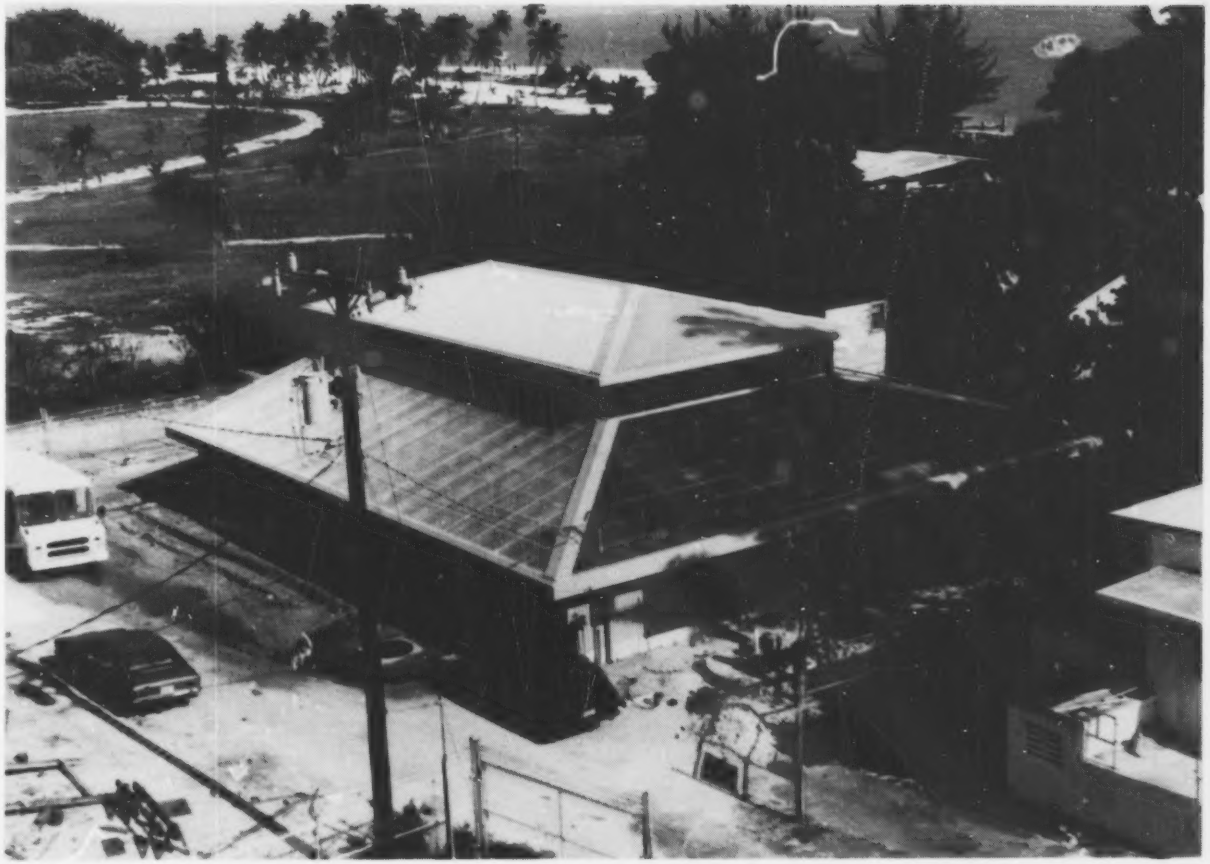

Figure 2. Exterior view of STABEL, looking in an easterly direction. The body of water in the upper part of the photograph is the Atlantic Ocean.

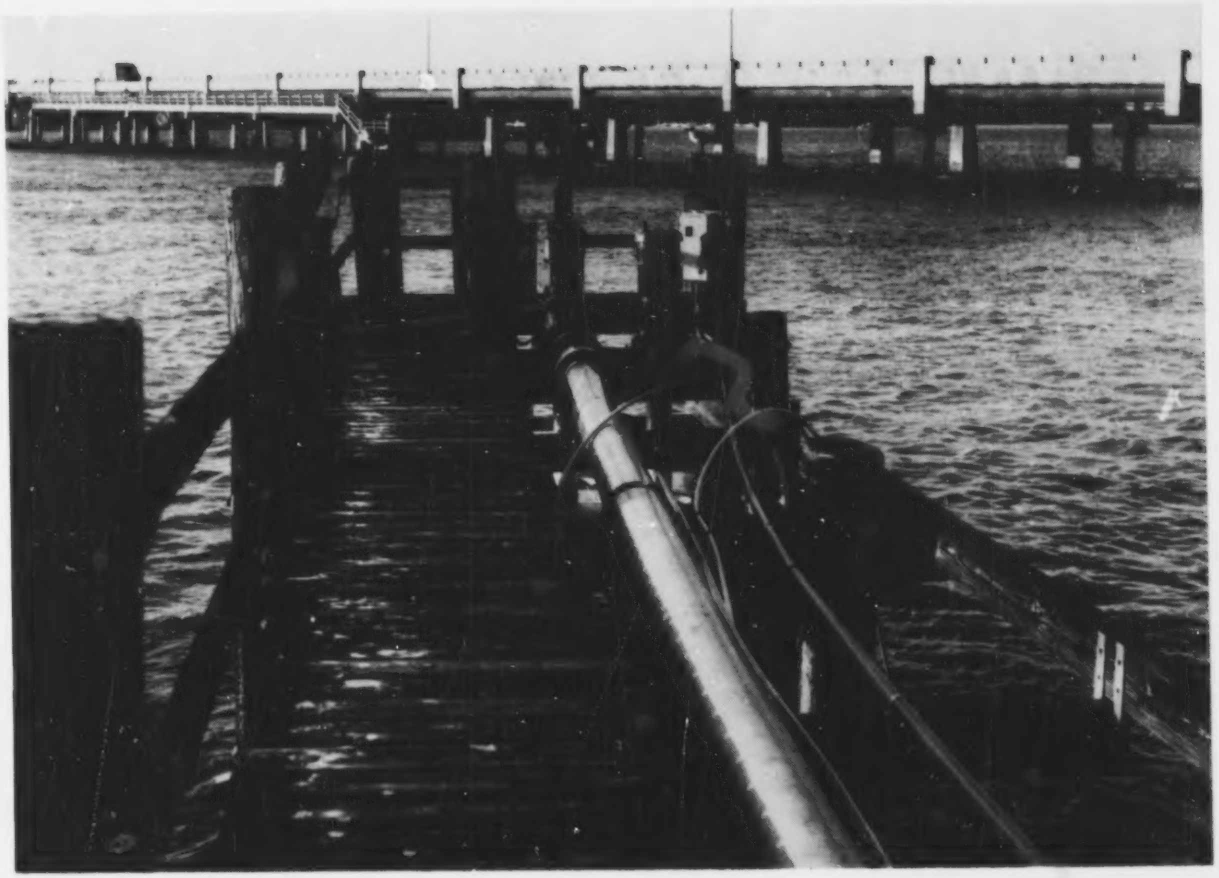

Figure 3. Saltwater supply pumps located on the end of the National Marine Fisheries Service pier. The pier is situated in Bear Cut, which separates Virginia Key and Key Biscayne.

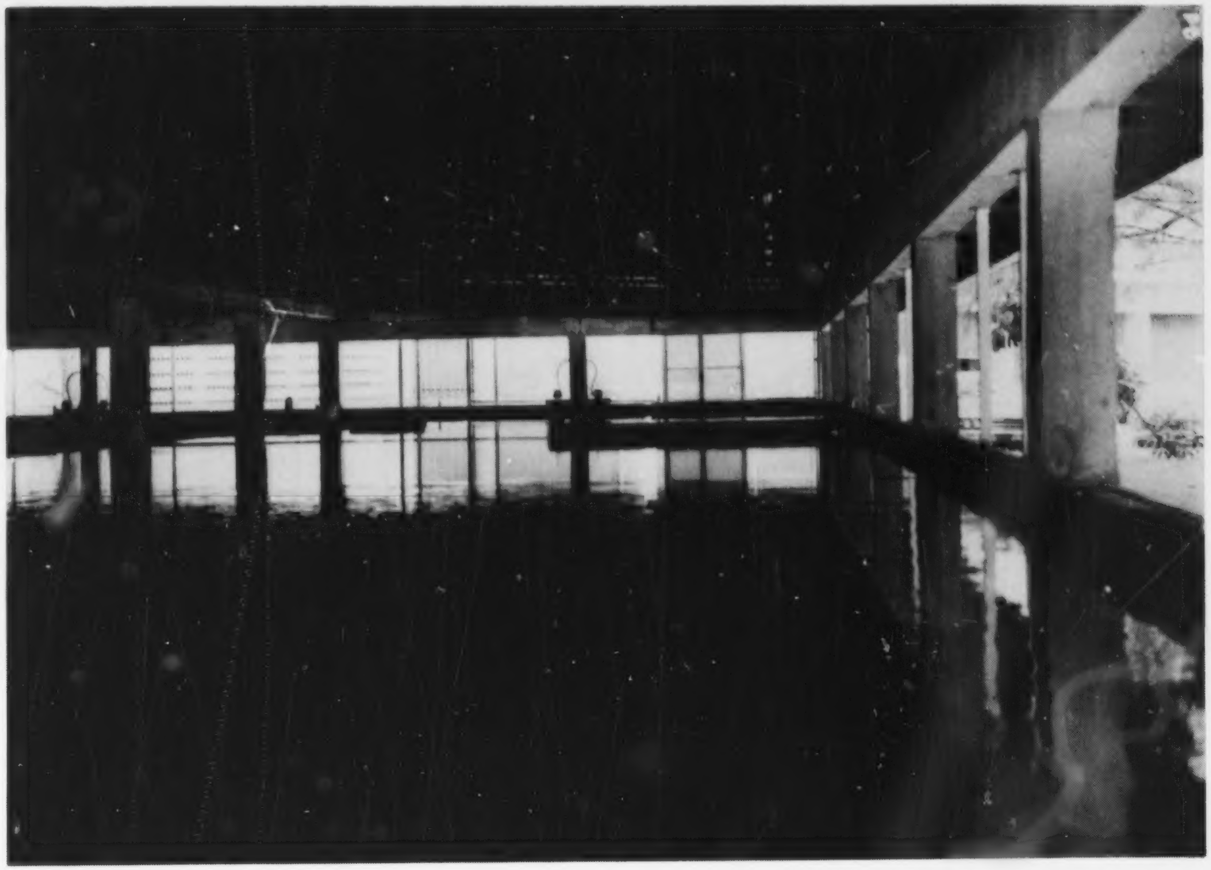

Figure 4. View of the interior of the salt water settling tank, used to settle all incoming seawater to STABEL.

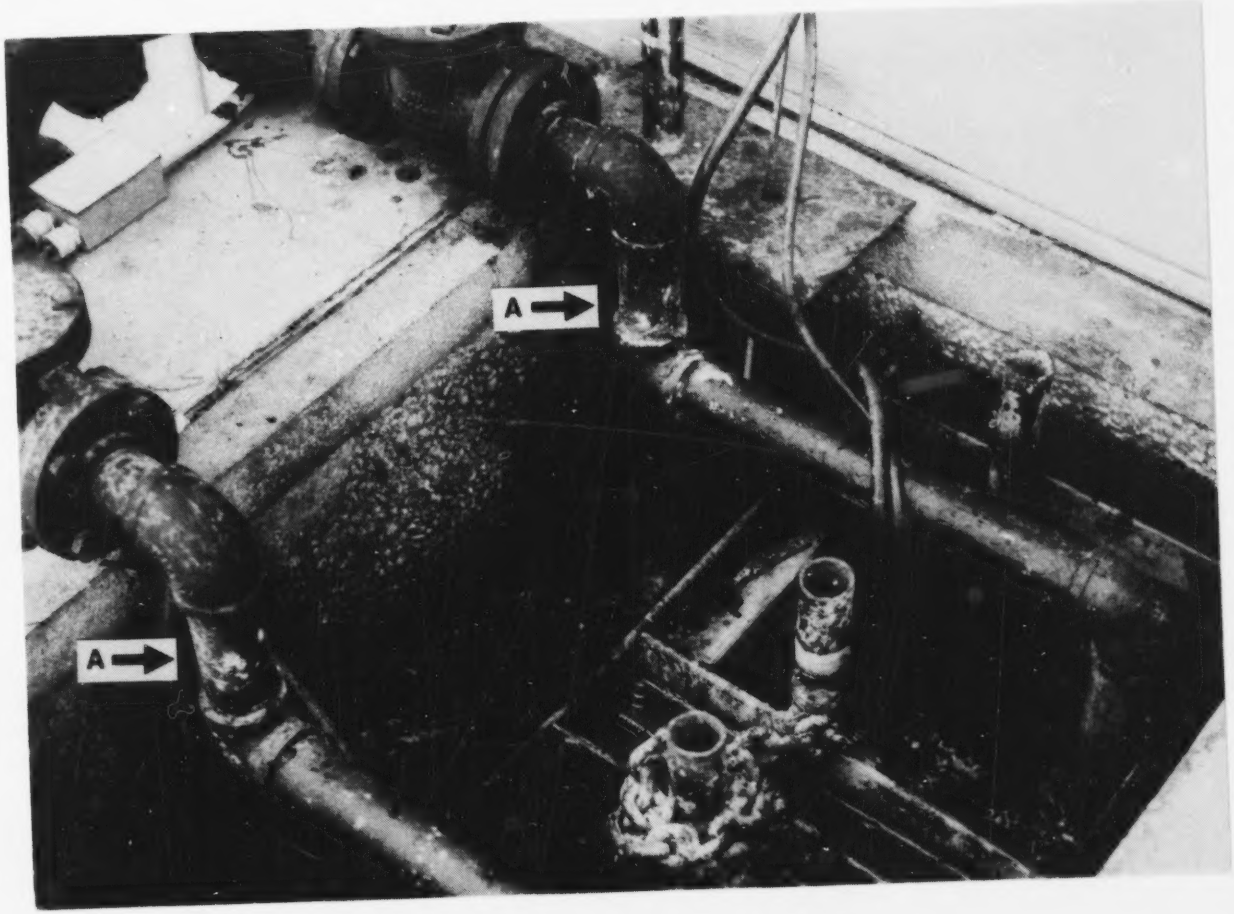

Figure 5. View looking down into the Virginia Key Sewage Treatment Plant grit chamber. The two output pipes (A) from the submersible grinder pumps are used to supply untreated wastewater to STABEL.

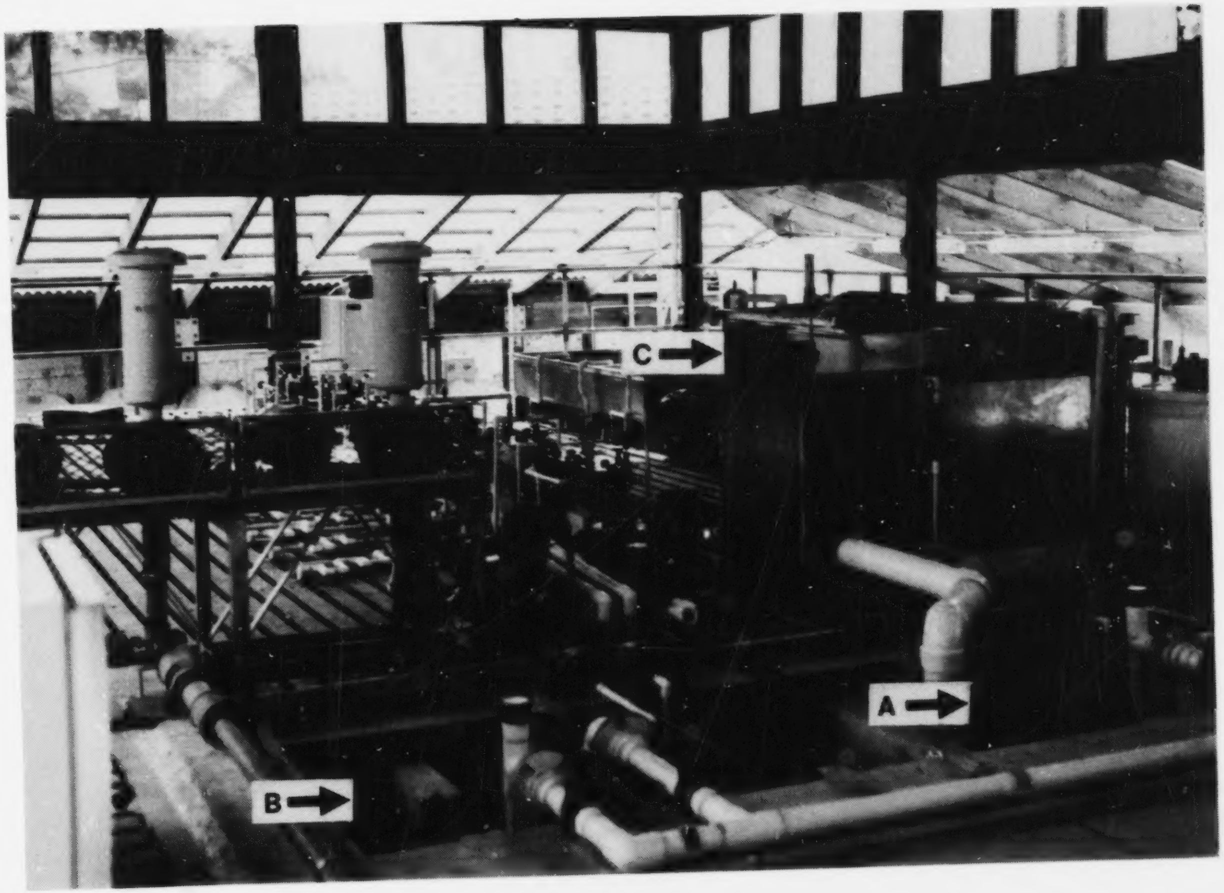

Figure 6. The upper level of STABEL (view is to the southwest). Arrows indicate the primary settling tank (A), the secondary settling tank (B), and the chemical flocculation tank (C).

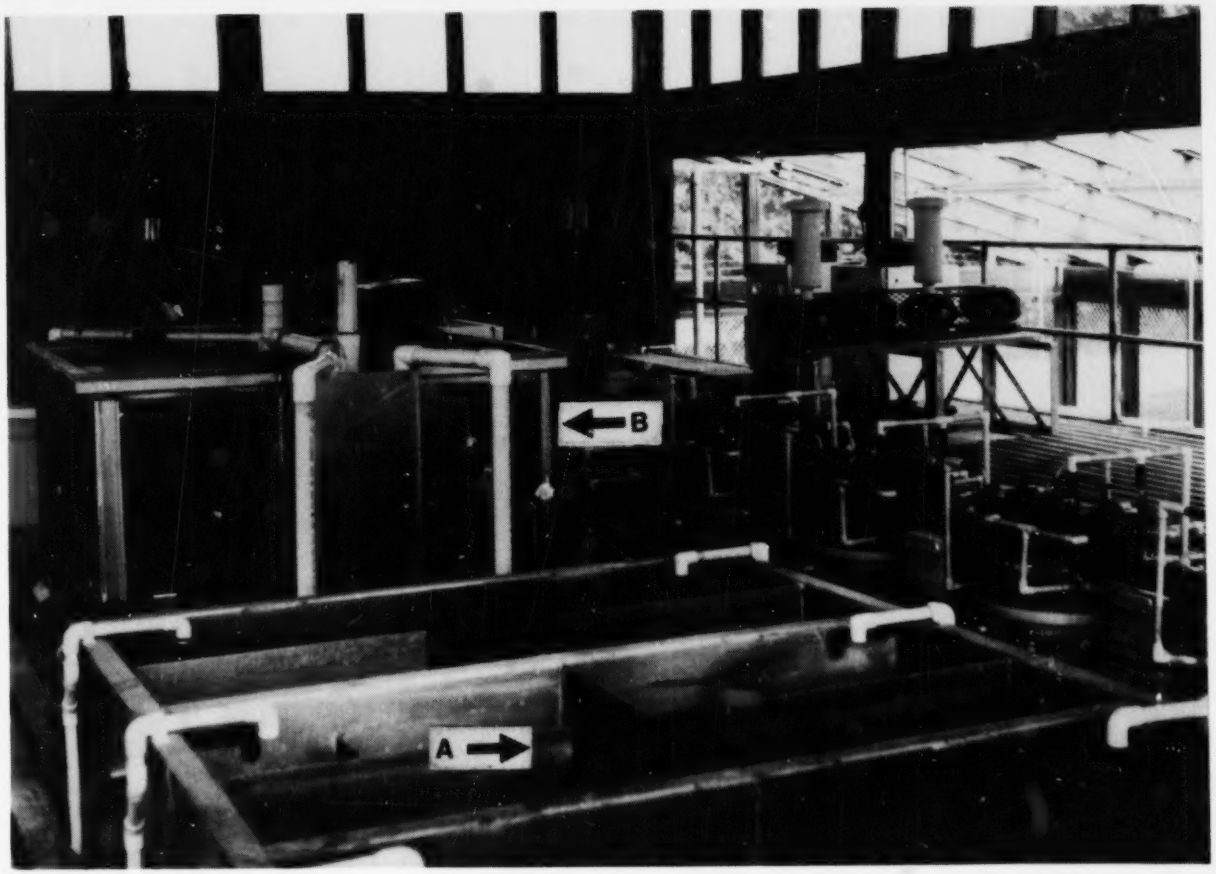

Figure 7. The upper level of STABEL (view is to the northeast). Arrows indicate the six detention tanks (A) and the chemical flocculation tank (B). The treated wastewaters in the three detention tanks on the left receive chlorination.

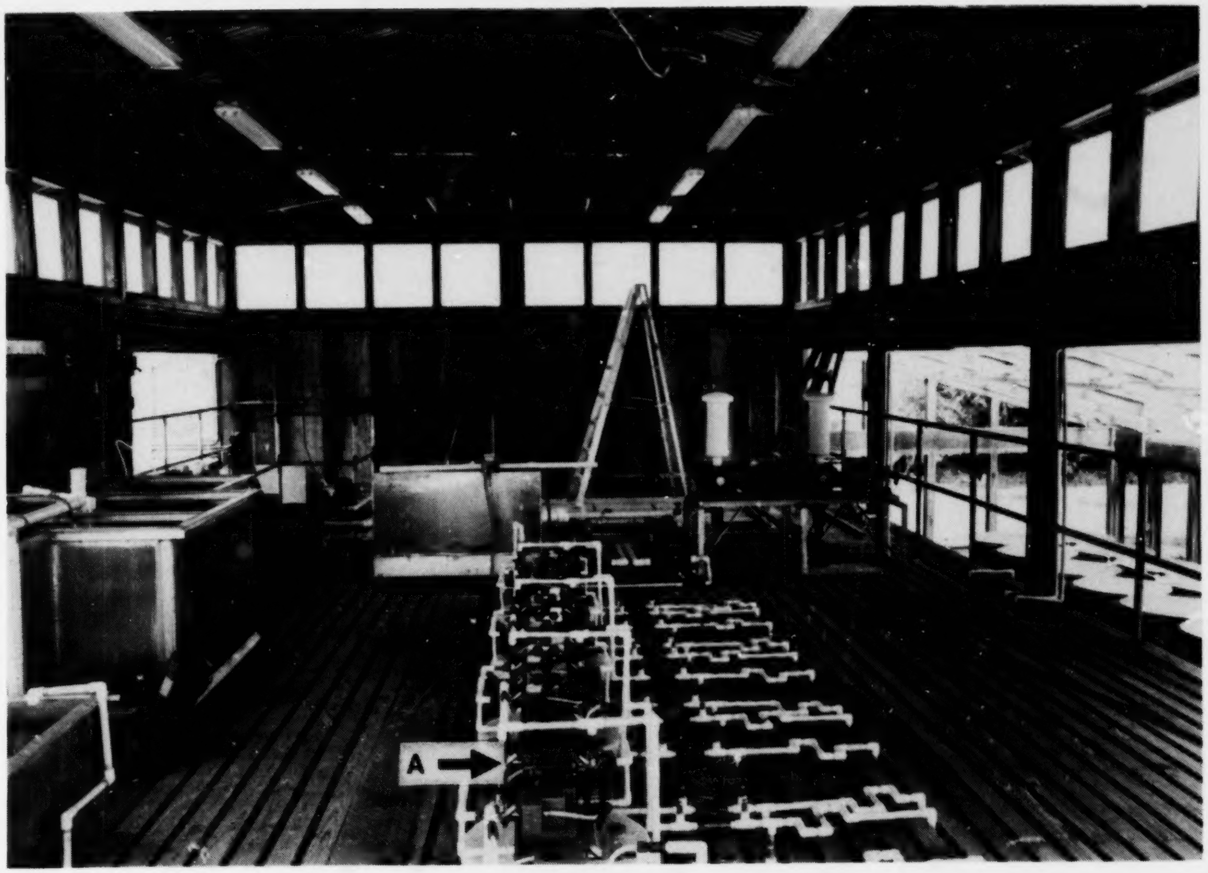

Figure 8. The upper level of STABEL (view is to the northeast). The arrow indicates the bank of adjustable metering pumps (A) used to inject the treated effluents into the seawater pipelines. The piping system located to the right of the pump is used to mix the seawater and effluent. The piping also has connection ports for inline flowmeters which are used to adjust the flows.

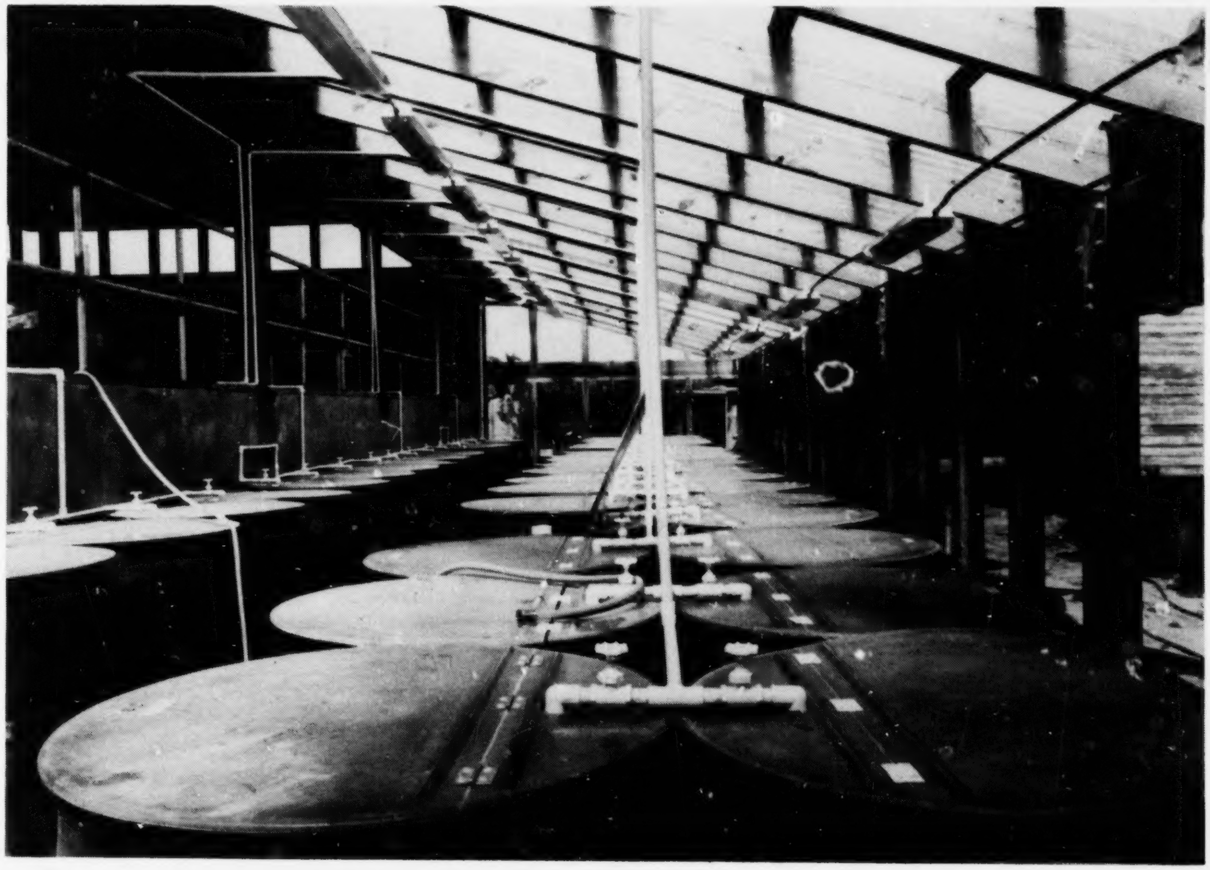

Figure 9. View of the eastern ground level side of STABEL, showing the 100 liter bioeffects tanks (grouped in fours) and their input plumbing.

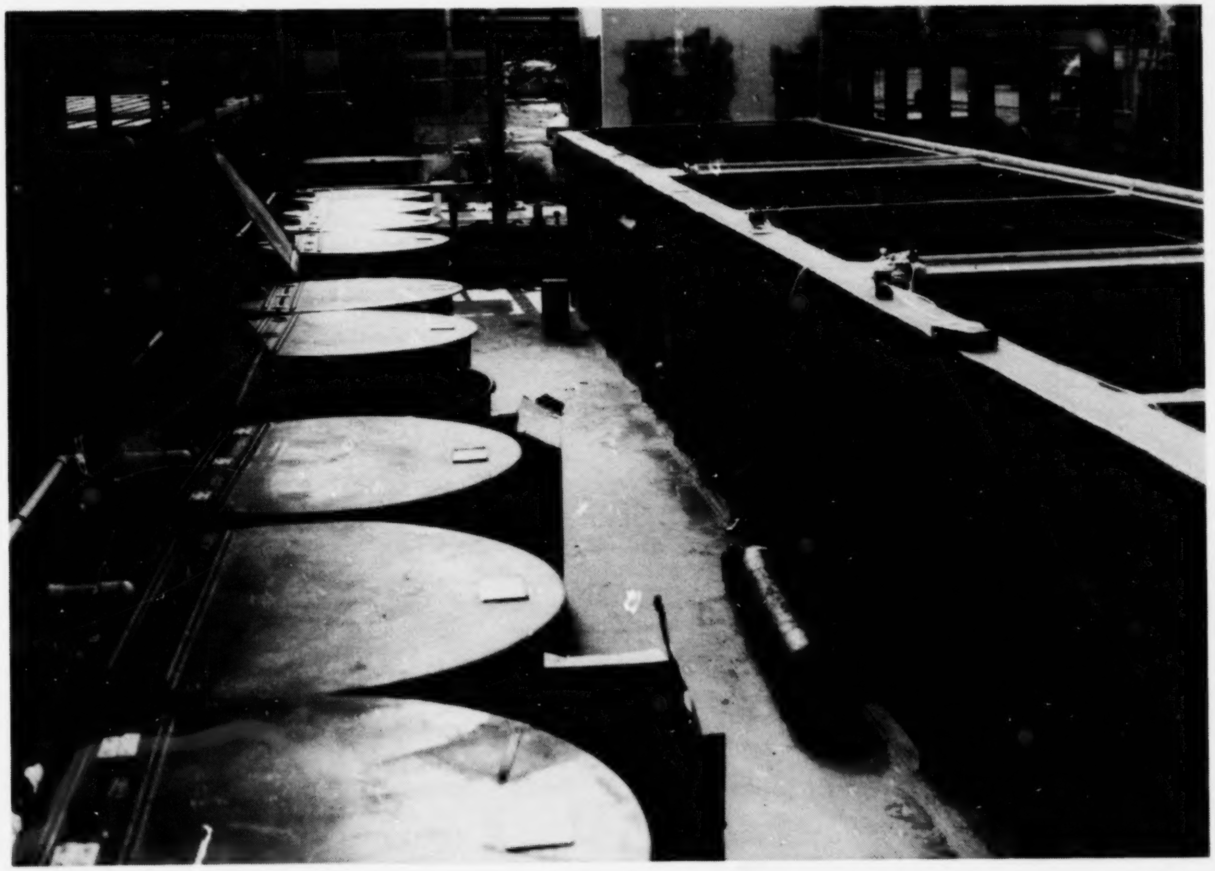

Figure 10. View of the western ground level side of STABEL, showing the 100 liter bioeffects tanks (left), and the large concrete holding tanks (right). These concrete tanks, which receive only seawater, are used to hold large quantities of test animals and acclimate them to the test facility.

is the largest treatment facility using ocean outfall disposal in Florida. On the average the Virginia Key sewage treatment plant processes 4.5 x 10^8 liter/day (120 mgd) of wastewater. The raw wastewater is taken from the entrance of a large grit chamber (Figure 5) and supplied to STABEL via a force main and submersible grinder pumps. The grinder pump assembly consists of two alternating pumps, each capable of delivering 210 liter/min (55 gpm). The raw wastewater enters the laboratory by a splitter box allowing the flow into the sewage treatment plant to be controlled. Any portion of the wastewater can be channeled through the plant or allowed to waste, thus being returned to the Virginia Key Sewage Treatment Plant. The wastewater is processed using primary settling, chemical flocculation of the primary settled wastewater, and activated sludge biological secondary settling. The chemical flocculation unit adds a known amount of the flocculant (aluminum sulfate) via a controllable process pump to the primary settled wastewater.

Wastewaters receiving primary settling, chemical flocculation, and activated sludge biological secondary settling (Figure 6) are sampled by rate-controllable process pumps which deliver a given wastewater to a pair of contact tanks. One of the pair receives chlorination and the other does not (Figure 7). This yields six different treatment processes: primary settled, chlorinated and non-chlorinated; chemical flocculation, chlorinated and non-chlorinated; and activated sludge biological secondary settled, chlorinated and non-chlorinated.

The chlorination of the treated effluents is done by an automated process which continuously monitors the residual chlorine content of the wastewater using amperimetric methods. Additional details are presented in Appendix D. The residual chlorine sensing unit controls the chlorine supply pump, thus theoretically keeping the residual chlorine at a constant, predetermined concentration (1 ppm for the STABEL bioeffects experiments).

The six treated wastewater effluents are then gravity fed to 95-liter (25-gal) holding tanks to simulate the passage through and residence time in the ocean outfall pipeline. The effluents are then pumped by rate-controllable process pumps into a seawater pipeline with a known flow rate (Figure 8). Controlling the rate of flow of seawater and the pumping rates of the effluents allows for an almost infinite set of dilutions. The effluent-seawater mixture is passed through a mixing chamber and then into 380-liter (100-gal) fiberglass bioeffects tanks containing the organisms for exposure (Figure 9). The system as presently designed takes the six effluents, dilutes each effluent to three different levels and replicates each of the dilutions in four of the bioeffects tanks.

STABEL contains eighty 380-liter (100-gal) bioeffects tanks. Seventy-two are used for seawater/effluent mixtures.

Eight are control tanks receiving only seawater. In addition, eight 5,030 liter (1,330 gal) concrete stock tanks (Figure 10) are available to hold and acclimate test organisms before use. All the tanks, bioeffects and stock, contain a modified siphoning system which assures a continuous turnover of seawater at all levels of the tank.

Seawater for the STABEL is supplied by a redundant pumping and settling system. Two alternating vertical turbine pumps draw seawater from screened intakes (Figure 3) situated approximately 2.5 m above the bottom and 50 m off Virginia Key in Bear Cut, which separates Virginia Key and Key Biscayne. The seawater is pumped into a redundant settling tank (Figure 4). The tank contains two 178 sq m (1,920 sq ft), three compartment settling tanks. The seawater, after passing through the settling tank, is pumped via two 190 liter/min (50 gpm) impellor pumps to STABEL. The redundant system, two turbine pumps, two settling tanks and two pairs of impellor pumps allows the seawater supply system to malfunction, be repaired, cleaned, etc., without any interruption in supply to STABEL.

As mentioned earlier, the STABEL facility is a closed system; all wastes, untreated effluents, treated effluents, sludge, and seawater/effluent mixtures are gravity-fed to a lift station with a redundant pumping system and pumped back to the Virginia Key Sewage Treatment Plant by way of a large wastewater force main.

The entire laboratory was equipped with a security, fire and mechanical breakdown detection system. Seawater, lift station and grinder pumps are all equipped with thermal detectors in addition to the routine electrical overload circuits. The submersible grinder pumps have water leak detectors for the motors and float and pressure sensors, and audio alarms that indicate a loss of supply of wastewater or blockage in the force main. The seawater pumps are controlled by a float system sensing when the settling tanks need filling and emergency shutoffs in case the supply of seawater is interrupted for any reason. If any malfunction, loss of electrical power, loss of seawater, etc., is detected, the alarm system is tripped which automatically phones an answering service which then notifies the correct project personnel. The alarm system also can detect fire and a breach of security in addition to equipment malfunction. The system is capable of differentiating between fire, security and equipment malfunction, thus giving project personnel foreknowledge of what action should be taken. This security system was installed to lower overall manhours and costs needed to maintain STABEL.

The building encompassing the STABEL facility (Figure 2) is of wood and concrete construction with steel supporting beams. The building is constructed on three levels. The above ground (upper) level contains the sewage treatment plant, contact tanks, dilution pumps and control panels. The ground level contains eight concrete stock tanks, eighty 380 liter (100 gal) bioeffects tanks, restroom and workbench/sink area. The below ground area contains the chlorinators, refrigerators, freezers, control valves and storage area. This below ground area also functions as an emergency overflow reservoir capable of containing 3 x 10^4 liter (7,929 gal) of wastewater/seawater before any spillage into the surrounding environment can occur. The building is screened on three sides and closed on the fourth. The raised center section of the building is covered by preformed aluminum roofing. The areas covering the bioeffects and stock tanks are preformed fiberglass roofing with a light transmission of approximately 88 percent. The transparent roofing over the bioeffect tanks and stock tanks allows the optional use of photosynthesizing organisms for research purposes.

STABEL OPERATING PARAMETERS

An operator (Jack Baird, Water and Wastewater Systems, Inc., Miami) with extensive experience in operating sewage treatment plants was retained to bring the STABEL facility into as similar an operative mode as possible (regarding primary and secondary treatment) with the Virginia Key Sewage Treatment Plant. Average values (obtained from monthly means) for common wastewater constituents measured during 1980 in (raw sewage) influent and (biological secondary) effluent at the Virginia Key STP are listed in Table 1. Frequent (often daily) contact was made by Mr. Baird with fellow operators at the Virginia Key plant to ascertain the current status of the wastewater influent measured there, in an effort to "fine-tune" the STABEL facility. Average values for wastewater constituents measured in influent and wastewaters treated by the primary, chemical flocculation, and biological secondary processes at the STABEL facility between March and October, 1980 are listed in Table 2. Comparison of these results for total non-filterable residue (total suspended solids) and BOD in both the influent and biological secondary wastewater with corresponding values for the Virginia Key plant (Table 1) reveals no significant differences for these two important water quality constituents. This indicates that during 1980 the STABEL facility was receiving a reasonably representative sample of raw sewage influent from the Virginia Key plant, and that at least the biological secondary treatment process of STABEL was representative of the corresponding process at the Virginia Key plant. On the average, this secondary treatment removed 70 percent of the total suspended solids and 84 percent of the BOD measured in the influent to the STABEL facility.

Based on the experience gained in operating the STABEL facility, the estimated (1980) cost of maintaining this type of experimental facility was approximately $86,000 (Table 3).

TABLE 1. AVERAGE (± 1 STD. ERROR) VALUES[a] FOR WATER QUALITY CONSTITUENTS MONITORED IN INFLUENT (RAW SEWAGE) AND FINAL (BIOLOGICAL SECONDARY) EFFLUENT AT THE VIRGINIA KEY SEWAGE TREATMENT PLANT DURING 1980 (mg/liter)[b]

Constituent	Raw Sewage Influent	Bio. Second Effluent
Total Residue	1930 ± 57	1840 ± 54
Total Fixed Residue	1360 ± 36	NA[c]
Total Volatile Residue	570 ± 15	NA
Total Non-Filterable Residue[d]	160 ± 8	45 ± 4
pH (units)	7.1 ± 0.0	7.2 ± 0.0
Biochemical Oxygen Demand	120 ± 4	41 ± 4
Chemical Oxygen Demand	NA	120 ± 3
Organic Nitrogen	NA	8.7 ± 0.5
Ammonia Nitrogen	NA	12 ± 0.5
Nitrite Nitrogen	NA	0.6 ± 0.2
Total Phosphate	NA	4.8 ± 0.4
Ortho Phosphate	NA	3.7 ± 0.2
Total Coliform (10^3 MPN)	NA	22,900 ± 4,400
Fecal Coliform (10^3 MPN)	NA	4,700 ± 800

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[a] From monthly mean values listed in Tables 32-34.

[b] Unless otherwise indicated.

[c] Not analyzed.

[d] Total suspended solids.

TABLE 2. AVERAGE (+ 1 STD. ERROR) VALUES[a] FOR WATER QUALITY CONSTITUENTS MEASURED IN INFLUENT (RAW SEWAGE) AND EFFLUENTS OF THE PRIMARY SETTLING, CHEMICAL FLOCCULATION, AND BIOLOGICAL SECONDARY TREATMENT PROCESSES AT THE STABEL FACILITY DURING 1980

Constituent	Raw Sewage		Primary		Chem. Floc.		Bio. Second.	
Units: mg/liter[b]	Ave.	S.E.	Ave.	S.E.	Ave.	S.E.	Ave.	S.E.
Total Residue	1420	100	1460	130	1540	100	1660	110
Total Volatile Residue	330	20	300	20	250	20	280	20
Total Non-Filterable Residue[c]	160	30	160	60	76	14	49	14
Volatile Non-Filterable Residue	53	22	55	15	26	5	19	6
Turbidity (NTU)	41	3	32	3	17	1	13	4
pH (units)	7.7	0.2	7.6	0.2	7.5	0.2	7.2	0.2
Biochemical Oxygen Demand	130	8	80	6	31	4	16	5
Organic Carbon	86	3	69	1	35	3	25	6
Ammonia Nitrogen[d]	16	1	16	1	14	2	9.4	1.8
Nitrite Nitrogen[d]	0.1	<0.1	0.2	0.1	0.2	0.1	2.0	1.5
Nitrate Nigrogen[d]	9.5	5.0	7.4	3.5	10	4	10	3
Ortho Phosphate[d]	1.2	0.3	1.2	0.3	0.4	0.1	1.0	0.3
Units: ug/liter								
Cadmium	5.6	1.1	3.9	1.2	<2	--	<2	--
Chromium	32	6	28	8	12	3	14	5
Copper	29	4	38	8	7.8	3.7	7.6	4.1
Lead	20	2	19	4	10	2	5.0	1.1
Zinc	140	16	130	22	28	9	27	11
Chlorinated Pesticides	0.6	0.2	0.7	0.5	2.7	1.5	1.1	--
Polychlorinated Biphenyls	1.9	0.9	8.1	7.3	0.5	0.2	4.5	--
Phthalic Acid Esters	73	15	100	30	160	34	110	29

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[a] From values listed in Tables 37-52.

[b] Unless otherwise indicated.

[c] Total suspended solids.

[d] Non-chlorinated wastewater.

TABLE 3. 1980 MONTHLY OPERATION AND MAINTENANCE COSTS FOR THE SEFOOS FACILITY

Description	$ per Month
Plant Supervisor and Operating Engineer	
5% of $26,000. p.a.	$1,300.
Fringe benefits at 15.6%	203.
Certified Class A Operator:	
Fixed fee	500.
Electricity (Florida Power & Light Company)	
Estimate based on operating history	300.
General Operation and Routine Maintenance	2,250.
Estimate based on operating history	
	$4,553.
Indirect Cost (at 58%)	2,641.
Operation and Maintenance Costs Per Month	$7,194.
Estimated Annual 1980 Operating Costs	$86,000.

SECTION 5

ENTEROVIRUSES AND INDICATOR BACTERIA FROM SUBMARINE OUTFALLS

INTRODUCTION

Present in these sewage effluents are pathogenic human enteric viruses whose fate in marine waters is not completely understood. Outbreaks of infectious hepatitis and nonbacterial gastroenteritis traced to the consumption of shellfish taken from polluted water indicate the importance of understanding the behavior of enteric viruses in marine waters.

Recent developments in virus concentration technology have made it possible to concentrate enteroviruses from 400 or more liters of turbid estuarine water (Payment et al., 1976) and from shellfish (Sobsey et al., 1975). However, evidence gathered by several investigators on the association of virus to solids indicates that sampling of the water alone may not give a true indication of the potential viral disease hazard (Bitten and Mitchell, 1974; Flora et al., 1975; Gerba and Schaiberger, 1975). Bacteriophages as well as enteric bacteria (Gerba and McLeod, 1976) have been found to survive for longer periods of time in marine water when adsorbed to clay than when held in free suspension (Bitten and Mitchell, 1974; Gerba and Schaiberger, 1975). Thus, sediments may act as a reservoir of enteric viruses, which could be resuspended by a number of natural and manmade events.

The development of a quantitative method for detecting enteroviruses in estuarine sediments has already been described (Gerba et al., 1977a). This section of the report is based on several improvements in the original method, and presents results of field studies on the occurrence of enteric viruses and bacteria in marine sediments and water in the immediate vicinity of three sewage outfalls, off south Florida. The section is based almost entirely on three publications of research resulting, at least in part, from the Southeast Florida Ocean Outfall Study (Gerba et al., 1977b; Edmond et al., 1978; Schaiberger et al., 1982).

PROCEDURES

The sampling and analytical procedures used in this study are described in detail by Gerba et al., 1977b; Edmond et al., 1978; and Schaiberger et al., 1982.

The Virginia Key outfall discharged approximately 55 mgd (1.77×10^8 liters/day) of secondary treated (activated sludge), chlorinated effluent from the Virginia Key Sewage Treatment Plant at a depth of 5 m (1,400 m off Virginia Key). This outfall now has been extended to a depth of 30 m (5,600 m from shore).

The Miami Beach outfall discharged 47 mgd (1.78×10^8 liters/day) of untreated chlorinated sewage at a depth of 44 m (3,600 m off Miami Beach). Use of this outfall was discontinued on October 14, 1980, when the flow was diverted to the Virginia Key system.

The Hollywood outfall lies in 33 m of water, discharging approximately 27 mgd (9.8×10^7 liters/day) of secondary treated chlorinated effluent.

RESULTS AND DISCUSSION

As evidenced by the data presented in Table 4, appreciable quantities of enteroviruses were isolated from surface seawater collected within 200 m of the discharge boil of untreated wastewater from the Miami Beach outfall. Also, several viruses were isolated from seawater samples collected above the chlorinated secondary outfall discharges at Miami and Hollywood. High seawater concentrations of fecal bacteria were also present in the vicinity of the Miami Beach outfall (Table 5). Chlorination of the secondary treated effluent at the Miami and Hollywood outfalls reduced the levels of fecal bacteria to almost below detectability. Although an appreciable amount of dilution occurs before the discharged Miami Beach sewage reaches the surface, viruses could still be detected at the surface boil using the newly developed concentration methodology.

The results of this study indicate that pathogenic human enteric viruses are detectable in and around non-treated sewage outfalls as well as in those secondarily treated, chlorinated sewage effluents. There was almost a 40,000-fold reduction in fecal coliforms in surface seawater collected within 200 m of the boils of the outfalls discharging treated chlorinated effluent, as compared to the one discharging raw sewage. In contrast, the average viral concentration reduction was only 10-100 times.

Outfalls discharging chlorinated secondary treated sewage resulted in a significant reduction in the amount of virus being discharged, although complete removal was not observed.

This study also included the analysis of surficial bottom sediments (top 3-4 cm) collected by divers in sterile containers, approximately 3 m from the ends of the three submarine sewage outfalls in the direction of the prevailing current.

TABLE 4. NUMBER OF ENTEROVIRUS ISOLATES (PFU[a] per 400 liters) FROM SURFACE SEAWATER SAMPLES COLLECTED WITHIN 200 M OF DISCHARGE "BOIL" OF THREE SOUTHEAST FLORIDA SUBMARINE SEWAGE OUTFALLS (AFTER TABLE 1 OF EDMOND ET AL., 1978)

Sampling Date (1977)	Hollywood (Chlorinated Secondary)	Miami Beach (Untreated)	Virginia Key (Chlorinated Secondary)
May	-	42	0
June	-	24	2
August	0	27	0
September	3	37	0
October	1	28	3
November	0	21	1

---

[a] Plaque forming units.

TABLE 5. CONCENTRATIONS OF BACTERIA (colonies/100 ml) IN SURFACE SEAWATER SAMPLES COLLECTED WITHIN 200 M OF DISCHARGE "BOIL" OF THREE SOUTHEAST FLORIDA SUBMARINE SEWAGE OUTFALLS (AFTER TABLE 2 OF EDMOND ET AL., 1978)

Sampling Dates (1977)	Total coliforms			Fecal coliforms			Fecal streptococci		
	VK[a]	MB[b]	H[c]	VK	MB	H	VK	MB	H
May	8	1.4×10^4	-	0	--	-	0	--	-
June	2	1.6×10^4	-	0	1.4×10^4	-	0	--	-
August	14	1.3×10^4	0	3	1.0×10^4	0	12	4.4×10^3	6
September	6	1.2×10^4	0	0	0.9×10^4	0	13	0.5×10^3	11
October	9	1.2×10^4	11	1	1.1×10^4	6	29	3.9×10^3	18
November	0	1.5×10^4	4	0	1.2×10^4	2	33	4.9×10^3	3

---

[a] M = Virginia Key

[b] MB = Miami Beach

[c] H = Hollywood

Table 6 presents results from a comparative study conducted during 1977 at the three outfall terminus sites.

On a volume-to-volume basis, greater concentrations of virus were present in the sediment than the overlying water at the three Florida outfall sites. The results shown in Table 6 indicate that concentrations of virus in the sediment are 3-4 orders of magnitude above those in the overlying water on a volume basis. This finding emphasizes the importance of effluent constituents which are concentrated on the seabed near coastal outfalls.

In view of concerns over the marine discharge of raw (untreated) sewage, the study around the Miami Beach outfall was extended (Shaiberger et al., 1982). The concentrations of enteroviruses and indicator bacteria measured in surface seawater collected within 200 m of the outfall boil between May 1977 and June 1978 are listed in Table 7.

A series of sediment samples was collected at various distances between the outfall pipe and the shore. The results shown in Table 8 indicate that the concentration of enteroviruses in the sediment was greatest at the submerged outfall pipe, and slowly decreased the greater the distance from the outfall pipe. Enteroviruses were isolated from sediments as close as 100 m from shore. To determine if enteroviruses could be detected in the surf at the bathing beach, a series of samples was collected along the bathing beach perpendicular to where the outfall pipe left the shore. Viruses were isolated from two of these samples (Table 9).

No indicator bacteria or viruses could be detected in water samples collected at distances greater than 200 m from the outfall. Indicator bacteria were also absent from the sediment at distances greater than 400 m and absent in the water and sediment at the bathing beach studied.

Enteroviruses isolated from the sediment included, in order of decreasing abundance, Polio 1, Coxsackie B4, B3, Echo 1, 6, 7, 26, 13 and 21.

Recent studies have indicated the presence of enteroviruses in the sediments of polluted coastal waters (Flora et al., 1975; Gerba et al., 1977a,b,c; LaBelle et al., 1980). Several of these studies indicated that viruses could be found in higher numbers in the sediment than in the overlaying waters on a volume basis. All of the studies on virus occurrence in sediments have been in shallow estuarine waters where water quality may be significantly different from the oceanic waters along the southeast coast of Florida.

As indicated in Table 7, appreciable quantities of enteroviruses and indicator bacteria were isolated from surface waters

TABLE 6. CONCENTRATIONS OF ENTEROVIRUSES IN THE VICINITY OF THREE SOUTHEAST FLORIDA SUBMARINE SEWAGE OUTFALLS (AFTER TABLE 2 OF GERBA ET AL., 1977b)

Site Location	Type of sewage discharged	PFU/100 liters*	
		Water	Sediment
Hollywood	chlorinated secondary	0.25	3160
Virginia Key	chlorinated secondary	0.50	2160
Miami Beach	untreated	7.3	9830

---

[a] Sediment samples were collected approximately 3 m from the outfall pipe terminus. Water samples were collected at the water surface within 200 m of the outfall pipe terminus. Water based on 400-liter sample size, sediment based on 1,200-ml sample size.

TABLE 7. CONCENTRATION OF ENTEROVIRUSES AND INDICATOR BACTERIA IN SURFACE SEAWATER COLLECTED WITHIN 200 M OF MIAMI BEACH OUTFALL "BOIL", 1977-78 (AFTER SCHAIBERGER ET AL., 1982)

Sampling Date	Enteroviruses[a] (PFU/100 liters)	Fecal coliforms per 100 ml (x 10^3)
May	42	--
June	24	14
August	27	10
September	37	9
October	28	11
November	21	12
December	30	36
January	44	41
February	27	55
March	36	19
May	59	12
June	41	18

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[a] Average of two samples collected within 200 m of outfall.

TABLE 8. CONCENTRATION OF ENTERIOVIRUSES AND INDICATOR BACTERIA IN SURFICIAL SEDIMENTS OFF THE MIAMI BEACH UNTREATED SEWAGE OUTFALL (AFTER SCHAIBERGER ET AL., 1982)

Date	Depth (m)	m from shore	Enteroviruses (PFU per liter)	Indicator Bacteria per 100 ml		
				Total coliforms	Fecal coliforms	Fecal streptococcus
11/2/77	41	3640	78	3200	1000	112
	27	3400	30	300	210	20
	18	3200	18	0	0	0
	3	300	ND	0	0	0
	1.5	100	2	0	0	0
3/21/78	41	3640	112	TNTC[a]	40,500	5200
	27	3400	49	1300	1000	140
	18	3200	21	0	0	0
	9	1500	14	0	0	0
	1.5	100	0	0	0	0
5/31/78	41	3640	96	3100	1300	110
	27	3400	42	220	890	61
	18	3200	23	0	0	0
	9	1500	20	0	0	0
	3	300	12	0	0	0
	1.5	100	0	0	0	0

---

[a] Too numerous to count.

TABLE 9. CONCENTRATIONS OF ENTEROVIRUSES AND INDICATOR BACTERIA IN SURFICIAL SEDIMENTS ALONG THE BATHING BEACH INSHORE OF THE MIAMI BEACH UNTREATED SEWAGE OUTFALL (AFTER SCHAIBERGER ET AL., 1982)[a]

Station	Depth (m)	Enteroviruses (PFU per liter)	Coliforms per 100 ml	Fecal Coliforms per 100 ml
1	1.2	0	0	0
2	0.9	15	0	0
3	1.5	30	0	0
4	1.5	0	0	0
5	1.5	0	0	0

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[a] Samples collected 400 m apart on beach 3.6 km from outfall pipe's discharge.

in the immediate vicinity of the outfall pipe, but indicator bacteria or enteroviruses could not be detected at distances greater than 200 m during the course of the study. In contrast, enteroviruses were detected in one of three sediment samples within 100 m of a bathing beach located inshore of the outfall. Analysis of the sediments for enteroviruses probably gives a better idea of the long term distribution of sewage effluent in the area, assuming that these viruses did not originate from other nearshore sources. Viruses settle out of suspension during the process and accumulate in the sediment material. Since viruses exhibit a longer survival time in sediments (LaBelle et al., 1980), viruses may have been deposited in the sediments several weeks previously. It is also possible that viruses which accumulate near the outfall pipe are transported shoreward by tides, currents or storm effects.

The concentrations of viruses found in the sediments were much higher than previously reported. LaBelle et al. (1980) reported concentrations as high as 48 PFU per 10 liters, and Gerba et al. (1977c) reported 332 PFU per 10 liters. In this study, the concentration at the outfall pipe ranged from 1,120 to 780 per 10 liters. On a volume basis, the concentrations of enteroviruses were over 2,000 times that found in the overlaying water at the outfall boil. In shallow coastal waters receiving secondary treated sewage, the concentration of enteroviruses in the sediment was found to be from 10 to 100 times that found in the overlaying water (Gerba et al., 1977c; LaBelle et al., 1980). A volume to volume comparison of virus concentrations may not be entirely valid since viruses are probably only present in the top few millimeters of sediment material, such has been observed for enteric bacteria in polluted waters (Van Donsel & Geldreich, 1971). Thus, their concentration in the first millimeter of sediment may be very high with few or no viruses present in the remaining sediment column.

As has been pointed out previously, microorganisms present in sediment material can be resuspended by currents, storms, boats, swimmers, dredging, etc. (Van Donsel & Geldreich, 1971). In shallow coastal waters, resuspension of the microorganisms could have a dramatic effect on the quality of the overlaying waters, but in deep coastal waters, such as near the outfall pipe in this study, the impact would not be significant because of dilution in the large volume of overlaying water. In addition, given the depth at the outfall, the chances of resuspension into the surface waters by storms, winds, boating, etc., would be greatly reduced. The significance of viruses in such material would be its eventual transport shoreward to bathing beaches or shellfish growing areas.

Indicator bacteria were isolated only in the immediate vicinity of the outfall while enteroviruses could be isolated from sediments as far as 3.6 km distant from the outfall. This suggests that the impact area of viruses on water quality was much larger than that of enteric bacteria and that bacteriological standards might not be reflective of the occurrence of enteric viruses. However, an alternate hypothesis is that the viruses occasionally measured near the bathing beaches came from an independent nearshore source. Failure to correlate the occurrence of enteroviruses with the presence of indicator bacteria has been previously recognized (Gerba et al., 1979), although in grossly polluted marine waters a correlation may exist (Petrilli & Flora, 1977; Vagel et al., 1981).

The implication of finding human pathogenic viruses in sediments from a public health viewpoint is difficult to assess, but enteric viral disease transmission by shellfish grown in sewage contaminated waters is well recognized (Gerba & Goyal, 1978), and gastroenteritis commonly associated with swimming in marine waters is suggestive of a viral etiology (Cabelli, 1981).

In summary, it has been shown that enteroviruses can occur in sediments relatively long distances from a raw sewage submarine outfall in the absence of viruses or indicator bacteria in the overlaying seawater.

SECTION 6

SUBMARINE OUTFALLS AND CORAL REEF ECOLOGY: FIELD AND PRELIMINARY LABORATORY STUDIES

INTRODUCTION

Stable community structure in coral reef areas can be viewed as a function of the architecture or physical structure of the habitat itself. The stability of this architecture is largely determined through the successful balance of biological and physical factors effecting $CaCO_3$ deposition and erosion. Organisms participating in this balance include stony corals, gorgonians and boring sponges. Because of their association with algal symbiants, stony corals and gorgonians are a significant component of overall benthic primary production. In southeast Florida, this greatly exceeds production in the water column. Stony corals are also the major depositors of $CaCO_3$ substrates, and thus are the primary builders of the physical habitat. Calcification by these organisms is dependent upon, and directly proportional to, the rate of primary production. In most instances, the two processes are inseparable. Balanced against primary production and associated $CaCO_3$ deposition is bioerosion. Sponges excavating $CaCO_3$ substrates are a major contributor to the destruction of primary reef structure. Under normal circumstances, when successfully balanced against depositional processes, this bioerosion will contribute to the formation of a more stable secondary structure through infilling with sediments and cementation. An imbalance can lead to the progressive deterioration of the habitat.

PROCEDURES

Quarterly surveys of coral, gorgonian, and sponge populations were conducted between December 1976 and April 1979 at five sites in the vicinity of the Miami Beach and Virginia Key municipal wastewater outfalls. Sites 1 and 2, inshore of the Miami Beach and Virginia Key outfalls respectively, were in 9 meter depths. Sites 3 (Virginia Key), 4 (Miami Beach) and 5 (Broward) were at the outfall depths (5 meters, 44 meters, and 33 meters, respectively). These surveys included measurements of nutrients (ammonia, nitrate, phosphate), primary production, coral calcification rate, and growth rate of corals and gorgonians. Comparative measurements also were made at control sites in the Florida Keys (Triumph Reef, Key Largo, Sambos), the Bahamas (Grand Bahama Island, Acklins and Crooked Islands) and Jamaica. Sampling in Florida and the Bahamas was quarterly, that in Jamaica at 6-9 month intervals. In addition, controlled laboratory studies were conducted at the Rosenstiel School of Marine and Atmospheric Science on the effect of dissolved organic compounds on coral calcification rates. Laboratory evaluations also were made on the bacterial content of sponge species collected from both outfall and control sites.

Concentrations of nutrients in samples of seawater (collected 3 m above the bottom) were determined following procedures specified by Strickland and Parsons (1972). The biological procedures used in this study generally follow those described by Chalker and Taylor (1975), Chalker (1976), and Duguay and Taylor (1978), and additional details will be described elsewhere (Dr. D. Taylor, personal communication).

RESULTS AND DISCUSSION

Field Studies

Biological Surveys--

Outfall study sites off Virginia Key, Miami Beach and Broward County exhibited typical encrusting patch-reef development found throughout the northern Florida Keys. These reefs occur as a secondary growth on limestone hardgrounds left on the submerged Pleistocene terrace that developed in this area. Their general form and appearance follows the ancient spur and groove pattern left by the terrace. North of Government Cut, (at the south end of Miami Beach), the fauna is best described as impoverished. This area includes the Broward and Miami Beach outfall sites. These features should be borne in mind when reviewing the field and laboratory data which follow. Because of obvious similarities in latitude and temperature regimes, control sites in the Florida Keys and Grand Bahama Island are most directly comparable. Similar terracing, and encrusting patch reef development, also occur in these areas.

A species list of corals, sponges and gorgonians observed in the outfall study areas is presented in Tables 10-12. The list also includes incidental observations on fishes (Table 13).

Nutrients--

Data on nutrients are summarized in Tables 14-16. In general, these compare well with previously published work in subtropical and tropical areas. Adjacent to the outfalls (sites 3, 4, 5), nutrients were somewhat more abundant. Median levels of ammonia and nitrate are 2-4 times those at the control sites, and there is a 2-fold elevation of orthophosphate at outfall site 4. Inshore sites 1 and 2 are not significantly different from control sites in the Florida Keys and Bahamas. Experimental studies reported below show corals and gorgonians are extremely efficient scavengers of ammonia, nitrate, and phosphate. Although the results of the field studies presented

TABLE 10. SPECIES LIST FOR STATIONS 1-5

Corals	Survey 1	Survey 2
Agaricia sp.	1-5	1-5
Mycetophelia sp.	4,5	4,5
Porites porites	1-5	1-5
P. ateroides	1-5	1-5
Manicina aureolata	1-3	1-3
Montastrea annularis	1-5	1-5
M. cavernosa	3-5	3-5
Oculina sp.	1-3	1-3
Madracis sp.	4,5	4,5
Dichocoenia stoksii	1-5	1-5
Eusmilia fastigiata	1-3,5	1-3,5
Mussa angulosa	2,5	2,5
Meandrina meandrites	1-3	1-3
Siderastrea siderea	1-5	1-5
S. radians	1-5	1-5
Acropora cervicornis	3	3

TABLE 11. SPECIES LIST FOR STATIONS 1-5

Gorgonians	Survey 1	Survey 2
Xestospongia muta	4,5	4,5
Verongia archeri	1-5	1-5
Verongia fistularis	1-5	1-5
Verongia longissima	1-5	1-5
Haliclona rubens	1-3,5	1-3
Agelas sp.	1-5	1-5
Iotrochota birotulata	1-3	1,2,4,5
Gelloides areolata	1-3	1-3
Cliona deletrix	1-5	1-5
Mycale laevis	1-5	1-5
Siphonodyction sp.	1-5	1-5
Verongia gigantea	1-5	1-5
Micale sp.	1-5	1-5
Ircinia strobilina	2,3	1-4
Ircinia sp.	1-5	1-5
Spongia sp.	1-5	1-5
Ectyoplasia ferox	4,5	4,5
Xestospongia sp.	1-5	1-5
Gelloides sp.	1-5	1-5
Callyspongia vaginalis	1-5	1-5
Ianthella sp.	1-5	1-5
Anthosigmella varians	2,4,5	2,4,5
Spheciospongia vesparium	1-5	1-5
Desmapsamma sp.	2	2
Callyspongia plicifera	1-3	2,3,4
Monanchora sp.	1-5	1-5

TABLE 12. SPECIES LIST FOR STATIONS 1-5

Sponges	Survey 1	Survey 2
Erythropodium polyanthes	1-5	1-5
Gorgonia ventalina	1-5	1-5
G. flabellatum	1-5	1-5
Briarium asbestinum	1-5	1-5
Muriceopsis flavida	2-5	1-5
Pseudopterogorgia bipinnata	1-5	1-5
P. americana	1-5	1-5
P. acerosa	1-5	1-5
Pterogorgia anceps	1-5	1-5
P. citrina	2-5	1-5
Iciligorgia sp.	1-5	1-5
Muricia muricata	4,5	4,5
M. laxa	4,5	4,5
M. elongata	4,5	1,4,5
Plexaurella nutans	1-5	1-5
P. dichotoma	1-5	1-5
Eunicia sp.	3,4,5	3,4,5
Eunicia mammosa	1-5	1-5

TABLE 13. INCIDENTAL OBSERVATION OF FISHES FOR STATIONS 1-5

Fishes	Survey 1	Survey 2
Thalassoma bifasciatum	1-3	1-3
Blennius marmoreus	1,2	1-3
Coryphopterus glaucofrenum	1,3-5	1-5
Pseudopeneus maculatus	2	2
Halichoeres poeyi	1,2	-0-
Equetus acuminatus	2,3	3
Haemulon plumieri	2	1-3,5
Halichoeres maculipinna	3	1-3
Equetus lanceolatus	3	-0-
Pomacanthus arcuatus (juv)	3	-0-
Cantherhines macroeros	3-5	2-5
Serranus tabacarius	4,5	4,5
S. baldwini	4,5	4,5
Sparisoma aurofrenatum	4-5	5
Holocanthus bermudensis	4	4,5
H. tricolor	5	4,5
Eupomacentrus partitus	5	3-5

TABLE 14. SEAWATER CONCENTRATIONS OF AMMONIA (ug-atom/liter) AT STUDY AND CONTROL SITES, DECEMBER 1967 - APRIL 1979

Site	12/76	4/77	6/77	9/77	12/77	4/78	6/78	9/78	12/78	4/79	Median
1	0.2	0.2	0.2	0.3	0.2	0.2	0.3	ND[a]	0.3	0.2	0.2
2	0.2	ND	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.2	0.2
3	0.7	0.6	0.6	0.7	ND	0.7	0.7	ND	0.6	0.5	0.6
4	0.6	0.6	0.6	0.6	0.7	0.7	0.8	0.8	0.7	0.7	0.7
5	ND	0.8	0.8	0.6	0.8	0.8	0.7	0.7	ND	0.6	0.8
Triumph Reef	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.2	0.2
Bahamas	0.2	ND	0.2	ND	0.2	ND	-	ND	-	ND	0.2

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[a] Not detected.

TABLE 15. SEAWATER CONCENTRATIONS OF NITRATE (ug-atom/liter) AT STUDY AND CONTROL SITES, DECEMBER 1976 - APRIL 1979

Site	12/76	4/77	6/77	9/77	12/77	4/78	6/78	9/78	12/78	4/79	Median
1	0.5	0.5	0.6	0.4	0.6	0.5	0.5	ND[a]	0.4	0.8	0.5
2	0.5	ND	0.5	0.5	0.6	0.5	0.6	0.6	0.5	0.6	0.5
3	1.0	0.9	0.9	0.9	ND	1.0	0.7	ND	1.1	1.0	1.0
4	1.1	1.0	1.0	0.8	0.8	0.9	0.8	1.1	1.0	1.0	1.0
5	ND	1.1	1.0	0.9	0.8	0.9	0.9	0.9	ND	0.9	0.9
Triumph Reef	0.3	0.5	0.4	0.4	0.4	0.5	0.5	0.3	0.4	0.5	0.4
Bahamas	0.3	ND	0.3	ND	0.3	ND	0.6	ND	0.3	ND	0.3

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[a] Not detected.

TABLE 16. SEAWATER CONCENTRATIONS OF ORTHOPHOSPHATE (ug-atom/liter) AT STUDY AND CONTROL SITES, DECEMBER 1976 - APRIL 1979

Site	12/76	4/77	6/77	9/77	12/77	4/78	6/78	9/78	12/78	4/79	Median
1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	ND[a]	0.1	0.1	0.1
2	0.1	ND	0.1	0.2	0.1	0.2	0.1	0.2	0.1	0.2	0.1
3	0.1	0.2	0.1	0.2	ND	0.1	0.1	ND	0.1	0.2	0.1
4	0.2	0.2	0.1	0.1	0.2	0.1	0.1	0.2	0.2	0.2	0.2
5	ND	0.3	0.1	0.1	0.1	0.2	0.1	0.2	ND	0.2	0.1
Triumph Reef	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Bahamas	0.1	ND	0.1	ND	0.1	ND	0.2	ND	0.1	ND	0.1

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[a] Not detected.

in Table 17 suggest that, on the average, a slight increase was observed for the in situ coral and gorgonian primary production rates for sites 3, 4, and 5 relative to those for site 1, there was no detectable increase in these outfall site values relative to those for site 2. Thus, it does not appear that the increased seawater nutrients measured nearest the outfalls substantially increases the primary production rates of these benthic species.

Primary Production--

Data on in situ primary production rates by corals and gorgonians are summarized in Table 17. These data span 18 months, and clearly show a strong seasonal variation in primary production, with the summer maxima exceeding the winter minima by about a factor of 1.5. Compared to the control sites (Triumph Reef, Bahamas, and Jamaica), benthic primary production was significantly lower at the outfall and inshore stations (sites 1-5). However, the data obtained in this investigation are insufficient to offer any insights as to the reason(s) for the lower primary production rates observed at the five stations within the study area.

Calcification--

In situ measurements of calcification by stony corals (Table 18) mirrored trends observed with primary production. Since the two processes are closely coupled metabolically, this is to be expected.

Growth Rates--

Short-term alizarin red staining of corals and growth ring analysis of gorgonians was conducted to determine long range growth rates at the study and control sites. Two corals, _Montastrea annulanis_ and _Oculina_ sp., and two gorgonians, _Erythropodium polyanthes_ and _Pseudopterogorgia bipinnata_, were monitored for an 18-month period beginning in June 1977. The results, calculated as rates of skeletal extension, are presented in Table 19. Coral extension was lower at all outfall and inshore sites studied. No apparent differences occurred with the gorgonians studied. However, the differences noted were small, and effects of temperature cannot be ruled out. A severe cold spell in 1977 may have greatly influenced the rates. At this time such studies appear to have only limited value.

Laboratory Studies

Organic Additions--

Additions of soluble organic compounds are known to suppress the rates of photosynthesis and calcification by corals (Chalker, 1975). These effects were studied in preliminary experiments using a range of known materials (Figures 11 and 12). These initial findings suggest the need for additional studies on the effect of soluble organics in wastewaters discharged near coral reefs.

TABLE 17. IN-SITU PRIMARY PRODUCTION RATES (g C/m^2 day) BY CORALS AND GORGONIANS AT STUDY AND CONTROL SITES, DECEMBER 1976 - JUNE 1978

Site	12/76	4/77	6/77	9/77	12/77	4/78	6/78
1	3.9	5.0	6.5	6.6	4.2	5.0	6.6
2	6.3	6.8	7.0	7.2	5.6	6.2	7.0
3	5.6	6.5	7.2	7.2	5.6	6.0	7.4
4	5.2	6.5	6.8	7.0	5.8	6.9	7.0
5	4.8	6.0	6.5	6.8	4.4	6.3	7.3
Triumph Reef	8.4	11.2	12.5	12.0	8.8	10.8	12.5
Bahamas	9.5	10.7	11.8	12.0	9.1	11.0	11.8
Jamaica	11.8	ND	15.5	ND	ND	14.8	16.2

TABLE 18. IN-SITU CALCIFICATION RATES (uM Ca^{+2}/hr) BY STONY CORALS AT STUDY AND CONTROL SITES, DECEMBER 1976 - JUNE 1978

Site	12/76	4/77	6/77	9/77	12/77	4/78	6/78
1	0.6	0.7	0.9	1.1	0.7	0.7	1.0
2	0.8	0.9	1.1	1.1	0.8	0.9	1.0
3	0.8	0.8	1.0	1.0	1.0	0.9	1.0
4	0.8	0.7	0.9	0.9	0.7	0.9	0.9
5	0.7	0.8	0.9	1.1	0.7	0.9	1.0
Triumph Reef	1.5	1.7	2.1	2.2	1.6	1.8	2.2
Bahamas	1.5	1.6	2.0	2.0	1.5	1.8	2.4
Jamaica	2.0	ND	2.5	ND	ND	2.3	2.7

TABLE 19. GROWTH RATES (mm extension/mo) OF CORALS AND GORGONIANS AT THE STUDY AND CONTROL SITES, JUNE 1977 - DECEMBER 1978

Site	*M. annularis*	*Oculina* sp.	*E. polyanthes*	*P. bipinnata*
1	0.2	0.1	0.1	0.1
2	0.2	0.1	0.1	0.1
3	0.1	0.1	0.1	0.1
4	0.1	0.1	0	0
5	0.1	0.1	0	ND
Triumph Reef	0.5	0.3	0.1	0.1
Bahamas	0.5	0.4	0.1	0.1

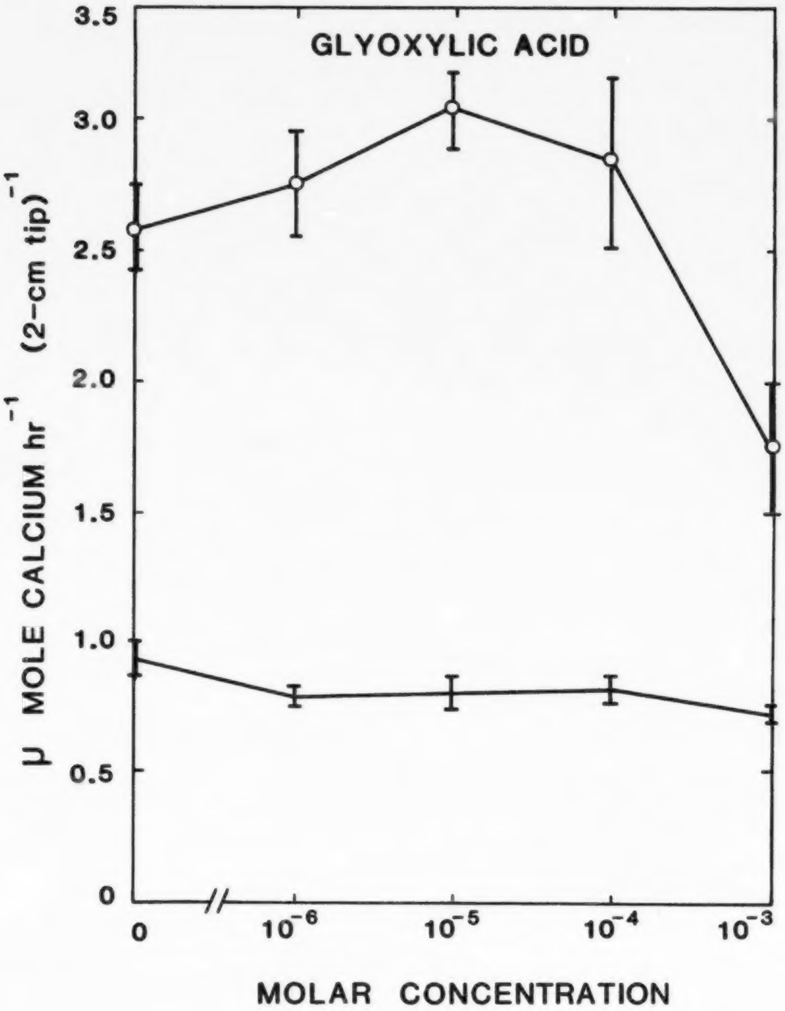


Figure 11. Effect of glyoxylic acid on the rate of calcification by Acropora cervicornis at 10,500 lux with (closed circles) and without (open circles) 10^{-5} M DCMU, or 3-(3,4-dichlorophenyl)-1,1-dimethylurea. Mean ± one standard deviation for four individuals.

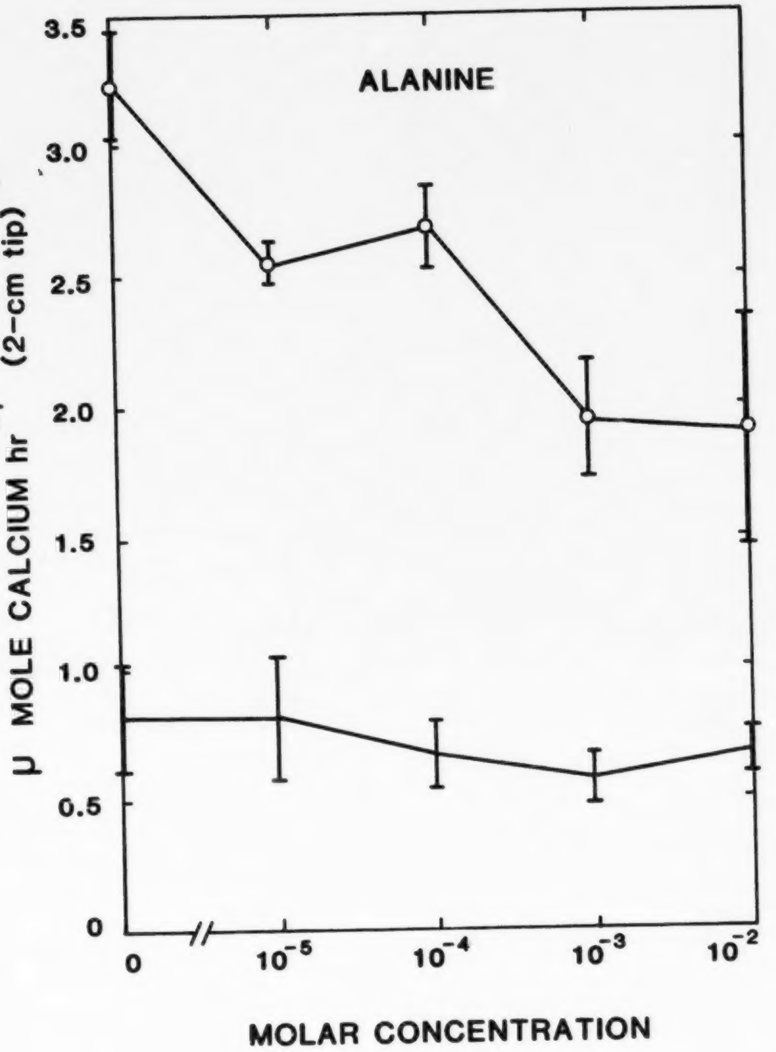


Figure 12. Effect of exogenous alanine on the rate of calcification by *Acropora cervicornis* at 10,500 lux (open circles) and in the dark (closed circles). Mean ± one standard deviation for five individuals.

Bacterial Flora of Sponges--

Two species of free-living, and two species of boring sponges were analyzed for bacterial content using discreet ($2\ cm^3$) tissue samples and various solid media. Samples were collected from outfall and control sites situated at Virginia Key and Key Largo. Seawater samples were also taken adjacent to the collecting area. Data are reported in Table 20, as total colony counts. Results indicate that sponges concentrate a rich bacterial population, and that individuals at outfall sites contain greater numbers of bacteria than those at control sites.

TABLE 20. BACTERIAL CONTENT OF SPONGE TISSUES (counts/cm^3) COLLECTED FROM THE VICINITY OF THE VIRGINIA KEY OUTFALL AND A CONTROL SITE OFF KEY LARGO

Virginia Key

	Water	Agelas	Verongia	Cliona	Siphonodyction
Blood Agar	180	3.4×10^4	3.0×10^4	2.6×10^4	2.8×10^4
Yeast/Peptone	1120	3.8×10^6	3.0×10^6	2.7×10^6	4.1×10^6
Casein	210	4.4×10^4	4.8×10^4	2.0×10^5	2.2×10^5

Key Largo

	Water	Agelas	Verongia	Cliona	Siphonodyction
Blood Agar	110	ND	ND	ND	ND
Yeast/Peptone	650	5.2×10^4	5.6×10^4	6.7×10^4	1.0×10^5
Casein	120	2.0×10^4	1.7×10^4	3.1×10^4	7.5×10^4

SECTION 7

MARINE FISHES EXPOSED TO SEWAGE EFFLUENT: FIELD AND PRELIMINARY LABORATORY STUDIES

INTRODUCTION

In preparation for the chronic toxicity tests on marine fishes planned for the STABEL facility, a number of activities described below were conducted.

LITERATURE SEARCH

A computerized search of NTIS, Environline, and Toxline Files was conducted using three lists of key words based upon the following subject area: (1) pathological effects of sewage pollution and sewage effluents on fish and shellfish, (2) acute and chronic bioassay procedures and bioassay laboratory design, and (3) effects of key toxicants of fish and shellfish with emphasis on chlorine, chloramines, ammonia, sulfide, major pesticides, heavy metals, hydrocarbons and surfactants. An additional survey provided extensive information on the biology, ecology and natural feeding habits of all species which were used in the sewage bioeffects experiments. The result was the retrieval of over 400 titles selected as having possible relevance to the SEFOOS project. These are listed in the Partially Annotated Bibliography (Appendix B).

OUTFALL AND CONTROL ZONE SURVEY

Preliminary studies of the health of fishes from Biscayne Bay have shown that gross external and internal pathology can provide an index of fish health (L. Udey, unpublished manuscript). Several fish species from the North Bay were observed to have a high incidence of severely split and eroded fins, fin hemorrhages, body hemorrhages and scale disorientation.

In the SEFOOS study, this approach was applied to the study of fishes frequenting the Virginia Key outfall (chlorinated biological secondary effluent) and the Miami Beach outfall (screening only), as well as three local control sites (Brewster Reef, Marker 23 off Cape Florida and at Soldier Key). Earlier studies of fishes caught on the Bay side of Soldier Key indicated that specimens from this area were in excellent health, displaying practically no disease symptoms. In the study discussed here, 34 fish were collected in 2-3 day "Z" trap sets at the three control sites during 1977. All except one fish was free of any external disease symptom; one fish had a hemorrhaged dorsal fin which appeared to be the result of trapping (Table 21).

Fish were also collected during 1977 in "Z" traps at the Virginia Key outfall (prior to its extension). The 51 fish from this outfall site were also free of external disease symptoms, with the exception of two Archosargus rhomboidalis which had moderate hemorrhages of the caudal fin (Table 22).

Fish could not be collected in traps at the Miami Beach outfall because of the strong currents in this area. (This also became a problem at the Virginia Key outfall when it was extended.) Sampling at the Miami Beach outfall, therefore, was limited to those fish which could be caught by hook and line. Of the seven fish examined, all but one appeared externally healthy (Table 22). This one fish had hemorrhaged pectoral fins.

Because gross external and internal pathology observations do not necessarily eliminate disease involvement, tissues were sampled from 23 selected fish collected in the vicinity of the outfall and at control sites for histopathology. Some of these specimens were processed. The most striking result of this histopathology investigation was the diversity of tissue structure and organization, not only among species but also among individuals. In particular the melanomacrophage centers in spleen, liver and kidney varied considerably both in degree of melanization and prevalence. Some grunts and sea bream from outfall sites showed 30-40 percent of splenic tissue so occupied. These centers may function as metabolic "dumps" and so may be directly related to contaminated environmental conditions. Moreover, they are reported to increase when Vibrio diseases are present, due to the increased breakdown of erythrocytes and bacterial phagocytosis. Age of the fish is also a factor.

Otherwise, as far as could be determined, there were no major differences between outfall zone and control site fish in the organs sampled. Parasitic invasion appeared minimal in all tissues. Gills were not hyperplastic although aneurisms were not infrequent even in control fish. These were probably the result of delayed deaths of the fish on shipboard. Necrosis and focal grannulomas were not seen.

Fish which had hemorrhaged fins showed no other histopathologic lesions resulting from bacteremia and viremia.

TABLE 21. EXTERNAL PATHOLOGY OF MARINE FISHES CAUGHT AT THREE CONTROL SITES IN BISCAYNE BAY (1977)

Common Name	Scientific Name	No. Normal No. Caught	No. Diseased; Condition
Brewster Reef			
Tomtate	*Haemulon aurolineatum*	4/4	0
Squirrelfish	*Holocentrus ascensionis*	4/4	0
Spotted goatfish	*Pseudupeneus maculatus*	3/3	0
Moray eel	*Gymnothorax* sp.	4/4	0
French grunt	*Haemulon flavolineatum*	3/3	0
White grunt	*Haemulon plumieri*	1/1	0
Bar jack	*Caranx ruber*	0/1	1 Hemorrhaged dorsal fin
Sand tilefish	*Malacanthus plumieri*	1/1	0
Rock hind	*Epinephelus adscensionis*	4/4	0
Cape Florida Channel			
Blue runner	*Caranx fusus*	2/2	0
Pinfish	*Lagodon rhomboides*	2/2	0
Lane snapper	*Lutjanus synagris*	1/1	0
Pigfish	*Orthopristis chrysoptera*	1/1	0
Soldier Key			
Nassau grouper	*Epinephelus striatus*	1/1	0
White grunt	*Haemulon plumieri*	1/1	0
Yellowfin grouper	*Mycteroperca venenosa*	1/1	0

TABLE 22. EXTERNAL PATHOLOGY OF MARINE FISHES CAUGHT NEAR THE SUBMARINE DISCHARGES OF THE VIRGINIA KEY[a] AND MIAMI BEACH MUNICIPAL WASTEWATER OUTFALLS (1977)

Common Name	Scientific Name	No. Normal / No. Caught	No. Diseased; Condition
Virginia Key Outfall			
Scrawled cowfish	*Acanthostracion quadricornis*	1/1	0
Ocean surgeon	*Acanthurus bahianus*	3/3	0
Sea bream	*Archosargus rhomboidalis*	30/32	2 Hemorrhaged caudal fins
Spiny puffer	*Diodon holocanthus*	1/1	0
Cubbyu	*Equetus umbrosus*	1/1	0
Tomtate	*Haemulon aurolineatum*	7/7	0
White grunt	*Haemulon plumieri*	1/1	0
Hogfish	*Lachnolaimus maximus*	1/1	0
Mangrove snapper	*Lutjanus griseus*	1/1	0
Fringed filefish	*Monacanthus ciliatus*	2/2	0
Spotted scorpionfish	*Scorpaena plumieri*	1/1	0
Miami Beach Outfall			
Gray triggerfish	*Balistes capriscus*	1/1	0
Saucereye porgy	*Calamus calamus*	2/3	1 Hemorrhaged pectoral fins
Sheepshead porgy	*Calamus penna*	1/1	0
Blue runner	*Caranx fusus*	2/2	0

---

[a] Prior to extension of outfall pipe.

PRELIMINARY LABORATORY STUDIES

Holding Success

In preparation for the fish bioeffects experiments in the STABEL facility, experimental feeding and holding studies were conducted on several potential test species. It was necessary to determine that all the candidate test species could be held for sufficiently long periods in the laboratory using flowing Bear Cut water (the dilution water or receiving water to be used on bioassay experiments). The SEFOOS investigators were able to hold fish of all species selected for periods in excess of three months, and held *Lagodon* sp. and *Lutjanus* sp. up to a year, maintaining them in a healthy state. This was achieved by providing flows which ensure that tank water was turned over at least 12 times per day, and by providing a proper diet.

Feeding Studies

Extensive testing of commercially available diets was conducted (Tables 23-25). Pinfish, mojarra, sheepshead minnow and killifish all responded similarily in their acceptance of the test diets. All four readily accepted several varieties of brine shrimp (*Artemia*), penaeid shrimp, mixed Pacific euphasiids, lobster eggs, mysus, and raw lobster. Mullet fed adequately only upon dried *Calanus plumchrus* (microplankton, Murex brand), Tetra Algae flakes, and frozen *Artemia*.

Retail price of these acceptable diets varied widely. Therefore, final selection of diet for bioassay experiments was based upon economics as well as acceptance. Of all the test diets, frozen brine shrimp was the best accepted and was fortunately the least expensive; this diet was selected as the primary diet for all species. Concentrations of potentially toxic trace metals in the two major brands of frozen brine shrimp used in these studies and listed in Table 26. Once a week feedings of penaeid shrimp or of Pacific euphasiids served to supplement brine shrimp feeding in order to stimulate feeding. It was found during feeding experiments that this supplemental feeding was essential when holding fish for extended periods.

Operations Procedure Manual

A detailed operational procedures manual was developed for use in the bioeffects facility, This manual was produced to ensure adequate control over all aspects of the SEFOOS bioeffects procedures, including control of infectious diseases. This manual is presented in Appendix C.

TABLE 23. FEEDING RESPONSE[a] OF SELECTED BIOASSAY FISH TO COMMERCIALLY FRESH, FROZEN, DRIED, AND FREEZE-DRIED DIETS

Diet	Cost[b] (dollars per lb)	Bioassay Fish				
		Pinfish (Lagodon sp.)	Mojarra (Eucinostomus sp.)	Mullet (Mugil sp.)	Sheepshead Minnow (Cyprinidon sp.)	Killifish (Fundulus sp.)
Frozen Brine Shrimp						
Living World Brand	6.00	4	3	1	4	4
Jungle Brand	0.65	4	3	1	4	4
Freeze-Dried Brine Shrimp						
Long Life Brand	20.91	4	3	2	3	3
Penaeid Shrimp Tails						
Fresh - Key West	1.00	4	3	0	3	3
Frozen - Key West	1.20	4	3	0	3	3
Ocean Plankton						
Frozen Pacific Euphausiids (Murex Brand)	19.92	4	2	1	4	4
Dried E. superba (Murex Brand)	63.79	0	0	0	0	0
Dried E. pacifica	73.28	4	2	0	2	2
Dried Calanus plumchrus (micro plankton - Murex brand)	47.68	0	0	3	1	1
Tubiflex Worms						
Freeze-dried (Long Life Brand)	113.50	0	0	0	1	1

---

[a] Rated 0-4; 0, least response; 4, greatest response.

[b] Retail price.

TABLE 24. FEEDING RESPONSE[a] OF SELECTED BIOASSAY FISH TO COMMERCIALLY AVAILABLE STERILE (GAMMA IRRADIATED) DIETS

Diet	Cost[b] (dollars per lb)	Bioassay Fish				
		Pinfish (*Lagodon* sp.)	Mojarra (*Eucinostomus* sp.)	Mullet (*Mugil* sp.)	Sheepshead Minnow (*Cyprinidon* sp.)	Killifish (*Fundulus* sp.)
Sterile Diets						
Gamma Foods, Inc.	13.52					
Fine mussel		2	1	0	2	2
Whole mussel		1	1	0	1	1
Lobster eggs		4	3	2	4	4
Mysus		4	4	2	4	4
Red plankton		1	2	2	2	2
Lance fish		4	0	0	2	1
Marine algae		1	1	1	1	1
Whole shrimp (1-2 cm)		2	1	0	2	1
Tang diet (oatmeal)		0	0	2	0	0
Bloodworm		3	2	1	2	2
Squid		2	0	0	2	1
Limpet		1	1	0	1	1
Razor clam meat		2	1	0	2	2
Raw lobster		4	2	0	3	3
Red daphnia		3	3	3	4	3
Vitamin supplement		1	1	1	1	1

---

[a] Rates 0-4; 0, least response, 4, greatest response.

[b] Retail price.

TABLE 25. FEEDING RESPONSE[a] OF SELECTED BIOASSAY FISH TO COMMERCIALLY AVAILABLE PELLETED AND FLAKE DIETS

		Bioassay Fish				
Diet	Cost[b] (dollars per lb)	Pinfish (*Lagodon* sp.)	Mojarra (*Eucinostomus* sp.)	Mullet (*Mugil* sp.)	Sheepshead Minnow (*Cyprinidon* sp.)	Killifish (*Fundulus* sp.)
Pelleted Diets						
Long Life Brand	5.16	1	0	0	1	1
Purina Trout Chow	0.60	1	0	1	1	1
Tetra Min Tablets	35.28	3	1	0	2	2
Flake Food						
Tetra Min	23.84	2	1	2	1	1
Tetra Marin	28.00	2	1	2	1	1
Tetra Algae	18.10	0	1	3	1	1
Tetra FD Menlo	44.64	1	1	1	1	1
Tetra Squid	27.09	1	0	0	1	1

---

[a] Rates 0-4; 0, least response, 4, greatest response.

[b] Retail price.

TABLE 26. METAL RESIDUES IN TWO DIFFERENT BRANDS OF FROZEN BRINE SHRIMP

Brand	Metal (ug/kg dry wt.)					
	Cd	Cr	Cu	Ni	Pb	Zn
Jungle	ND[a]	1,365	9,290	ND	ND	50,560
San Francisco	465	1,875	10,240	2,510	ND	43,325

---

[a] Not detected.

Preliminary Bioeffects Studies

The initial SEFOOS bioeffects experiments were conducted in the Glassel Building on the RSMAS campus to provide a background of preliminary data and operational experience, and preliminary response data. These experiments were performed on a very limited scale. The initial experiment, with Cyprinodon variegatus, was designed to compare 30:1 dilutions of chlorinated and non-chlorinated final effluent from the Virginia Key Sewage Treatment Plant. Sewage samples were collected once daily, retained four hours and metered into 120-l experimental tanks (one tank/treatment; 2 control tanks) each containing 28 C. variegatus individuals.

Sewage bioassays continued using two additional species, Eucinostomus argenteus (spotfin majarra) and Anchoa sp. (anchovy). The first species tested (Cyprinodon variegatus) was terminated when 100 percent of the fish (controls included) died of Vibrio infection resulting, in part, from insufficient water turnover (once/4.6 hrs). To correct this situation in subsequent assays, sewage and seawater flows were doubled while maintaining the same 30:1 dilution of chlorinated and nonchlorinated secondary treated sewage from the Virginia Key treatment plant.

Sewage was metered by a Harward peristaltic pump, which maintained a constant rate for the duration of the experiment. Sewage flow, however, had to be manually adjusted frequently because of the variation in pressure of the Glassell water system, so that an average of 800 ml/min (± 10 percent) was maintained.

The Eucinsotomus experiment used ten fish weighing an average of 1 g per experimental group, while the Anchoa experiment used eighteen fish, weighing an average of 1.4 g per group. San Francisco brand frozen brine shrimp were used exclusively as food in the Cyprinodon and Eucinostomus experiments while Jungle brand was used for the anchovies.

No mortality was observed during the 37-day Eucinostomus experiment. All fish in the experiment were treated with Furanase after collection to prevent nonspecific mortality like that which occurred in the previous bioassay experiment. Fish were held for 30 days after treatment before use in experiments. At the end of the experiment representative samples were preserved for histology and chemical residual analysis.

Examination of histologic specimens revealed no lesions or abnormalities of internal organs or gill epithelium. Residual analysis data for trace metals and pesticides are listed in Tables 27 and 28. No consistent pattern in residue levels were observed.

TABLE 27. METAL RESIDUES IN WHOLE FISH SAMPLED AT TERMINATION OF PRELIMINARY BIOASSAY EXPERIMENTS

Experimental Group	Metal (ug/kg dry wt.)					
	Cd	Cr	Cu	Ni	Pb	Zn
1. *Eucinostomus*						
Non-chlorinated	ND[a]	ND	2,230	640	ND	52,105
Chlorinated	ND	ND	225	55	ND	51,970
Seawater Control	ND	ND	695	ND	ND	79,490
2. *Anchoa*						
Non-chlorinated	ND	ND	1,205	10	ND	68,800
Chlorinated	ND	ND	1,680	ND	ND	103,820
Seawater Control	20	ND	2,065	365	ND	85,005

---

[a] Not detected.

TABLE 28. ORGANIC RESIDUES IN WHOLE FISH SAMPLED AT TERMINATION OF PRELIMINARY BIOASSAY EXPERIMENTS

Experimental Group	Straight Chain Chlorinated Hydrocarbons and PCB's	Clorinated Hydrocarbons with Epoxides	Plasticizers (phthalic acid ethers)
1. Eucinostomus			
Non-chlorinated	Trace of endosulfan I and DDA[a]	Large traces of several epoxides	Several
Chlorinated	Trace of endosulfan I and DDA	Traces of same epoxides as above	Several
Seawater Control	No pesticides	No epoxides	Very small trace
2. Anchoa			
Non-chlorinated	Trace of endosulfan I and DDA	Traces of different epoxides	Several
Chlorinated	Largest traces of endosulfan I and DDA	Traces of epoxides	Several
Seawater Control	Traces of endosulfan I and DDA	Traces of epoxides	Several

---

[a] Bis(p-chlorophenyl)acetic acid.

A 10 percent mortality was observed in each of the treatment groups (including controls) over the 40-day duration of the *Anchoa* experiment. Fish in this experiment were treated similarly to those in the *E. argentius* study.

All fish of this species examined histologically showed extensive microsporidasis of the pancreas. It is suggested that this was responsible for the initial mortality following collection and that this may make this species unsuitable for use as a bioassay organism. In addition, some anchovies from non-chlorinated sewage showed liver lipidosis and one from chlorinated sewage had what appears to be an invasive epithelioma on the tip of the nose. Protruding noses had been observed on several live anchovies in all three tanks. Occasional gill and foregut trematodes were also evident.

A limited analysis of the experimental streams sampled on the 30th day of the *Eucinostomus* experiment was completed (Table 29). A comparison of the waters receiving the chlorinated and non-chlorinated effluent streams did not reveal any important differences. Further, addition of these effluents to the seawater raised levels of most of the target nutrients no more than several-fold, with the exception of ammoniacal nitrogen (aqueous ammonia plus ammonium), which was elevated more than two orders of magnitude above the measured ambient seawater concentrations.

TABLE 29. ANALYSES OF EXPERIMENTAL TREATMENT STREAMS SAMPLED 30 DAYS INTO THE *Eucinostomus argentius* PRELIMINARY BIOEFFECTS EXPERIMENT

		Treatment Effluent		
Analysis	Units	Non-chlorinated	Chlorinated	Seawater
Salinity	0/00	32.70	33.20	34.30
D.O.	mg/l	5.60	5.02	5.60
Temperature	°C	24.5	24.7	24.0
Residual Halogens	mg/l	0.021	0.027	0.013
$NH_4^+ + NH_{3(aq)}$	mg/l	10.83	10.26	0.03
NH_3 (aq)[a]	mg/l	0.38	0.36	0.00
Total PO_4	ugA/l	2.00	6.06	1.05
Inorg.PO_4	ugA/l	1.40	2.45	0.50
NO_3^-	ugA/l	4.75	10.40	9.90
NO_2^-	ugA/l	0.05	0.10	0.10
SiO_3^-	ugA/l	7.00	10.00	3.00
pH	--	7.80	7.81	7.80

---

[a] Calculated from equations of Emerson et al. (1975)

SECTION 8

CHARACTERIZATION OF WASTEWATERS

INTRODUCTION

A meaningful interpretation of the results from the bioeffects studies conducted in the STABEL facility is dependent upon a sufficiently comprehensive description of the wastewaters to which the test organisms were exposed at various seawater dilutions. In this section, background information first is presented on the characteristics of the untreated (raw sewage) and treated (biological secondary) municipal wastewater of the Virginia Key Sewage Treatment Plant (STP), the source of the raw sewage treated in the pilot STABEL facility. Detailed results of several series of physical/chemical analyses on the STABEL wastewaters within the following major water quality categories:

General (e.g., solids, BOD, turbidity, pH)
Nutrients (e.g., nitrogen and phosphorus compounds)
Toxic Metals (e.g., cadmium, lead)
Synthetic Organics (e.g., chlorinated hydrocarbons, phthalates)

then are presented and discussed.

PROCEDURES

During the initial planning of the SEFOOS study, specific data related to operating characteristics of the Virginia Key STP were obtained from the Metro-Dade Water and Sewer Authority. Such data were needed to guide the design of the STABEL facility, and to provide background information for the experimental period. Thus, the 1980 records on the characteristics of the raw sewage received from the (original) Virginia Key STP were obtained and summarized. Corresponding data for the final (biological secondary) effluent from this plant also were tabulated to facilitate an evaluation of the efficiency of the STABEL biological secondary operation.

During the course of the bioeffects experiments, grab samples of the various types of wastewaters at the STABEL facility were collected and analyzed at the Rosenstiel School of Marine and Atmospheric Science. The procedures used for analysis of the general constituents were those specified by Standard Methods, 14th Edition (1975). For analysis of the nutrients, the samples were diluted and processed on an auto-analyzer following procedures described by Strickland and Parsons (1972). Procedures provided by the Environmental Protection Agency (1979) and Thompson (1977) were used for the concentration and trace analysis of selected toxic metals (by flame and carbon rod atomic absorption spectrometry) and synthetic organic compounds (by electron capture gas chromatography) respectively. A more detailed description of these analytical procedures is presented in Appendix D.

RESULTS AND DISCUSSION

Virginia Key Sewage Treatment Plant

Flow Rates--

Monthly time series data for 1977 Miami weather conditions and wastewater flow rates at the Virginia Key STP are presented in Table 30. These data show that the monthly highs in flow rate (approximately 120 million gallons per day) during the May and June peaks in rainfall are about double the monthly flow rate peaks (60-65 MGD) of the "dry" season (January-April). This suggests that rainwater draining into the sewage collection system contributes surface and near-surface groundwater contaminants to the wastewater treatment and submarine discharge systems. Corresponding data for most of 1980 (which had much more uniform rainfall) is presented in Table 31.

Water Quality Constituents--

Average monthly concentrations of general water quality constitutents and nutrients (where available) in the influent and final effluent of the Virginia Key plant are listed in Tables 32 and 33, respectively. Corresponding averages for total and fecal coliform bacteria measured in the final effluent and surface seawater samples from the outfall monitoring zone (stations locations shown in Figure 13) are listed in Table 34. Tables 35 and 36 present the limited available data for trace metals in the Virginia Key STP influent and effluent (1977 grab samples).

STABEL

Water Quality Constituents--

Values for the general water quality constituents measured in grab samples (March-October, 1980) of four types of STABEL wastewaters - untreated raw sewage influent and wastewaters treated by primary settling, chemical flocculation, and activated sludge (biological secondary) - are presented in Tables 37-40. These results show successive decreases in the median concentrations of the common water quality indicators Total Non-filterable Residues (Total Suspended Solids), Turbidity, Biochemical Oxygen Demand (BOD), and Total Organic Carbon (TOC) over this range of treatment, as summarized below:

TABLE 30. AVERAGE INFLUENT (RAW SEWAGE) FLOW RATES AND WEATHER CONDITIONS FOR THE VIRGINIA KEY SEWAGE TREATMENT PLANT DURING 1977

	Month											
	J	F	M	A	M	J	J	A	S	O	N	D
FLOW RATE (MGD)[a]												
Average	57.48	56.06	54.71	54.88	80.94	83.27	64.29	69.80	84.76	69.38	72.73	71.30
High	64.23	64.50	63.20	59.60	116.73	124.44	74.41	84.72	117.00	76.00	96.82	80.60
Low	51.40	51.20	48.90	46.70	48.30	59.60	53.80	59.40	71.00	60.00	63.40	64.64
TOTAL RAINFALL (INCHES)												
Virginia Key Plant	1.10	1.23	0.32	1.30	12.34	9.97	4.90	9.79	6.74	1.46	6.94	2.05
Miami Airport	1.42	2.09	0.91	1.90	15.82	12.41	5.23	8.55	7.04	1.25	5.94	2.55
TEMPERATURE (°F)												
Air	57	62	72	74	75	79	80	80	78	73	71	65
Sewage	78	79	80	81	80	82	83	82	82	82	81	80

---

[a] Million gallons per day, equivalent to 3.78×10^6 liters/day.

TABLE 31. AVERAGE INFLUENT (RAW SEWAGE) FLOW RATES AND WEATHER CONDITIONS FOR THE VIRGINIA KEY SEWAGE TREATMENT PLANT DURING 1980[a]

	Month											
	J	F	M	A	M	J	J	A	S	O	N	D
FLOW RATE (MGD)[b]												
Average	80.5	79.0	77.1	89.6	82.0	81.9	84.4	90.8	-	-	-	-
High	89.4	83.7	100.4	112.8	91.0	100.0	96.2	125.4	-	-	-	-
Low	76.0	72.5	69.4	77.1	75.7	73.7	72.5	78.1	-	-	-	-
TOTAL RAINFALL (INCHES)												
Virginia Key Plant	2.45	1.26	1.41	7.40	4.00	6.42	7.04	4.03	3.98	8.61	7.64	0.54
Miami Airport	2.48	0.88	3.16	10.20	3.80	3.02	9.40	11.32	5.60	6.05	3.47	0.19
TEMPERATURE (°F)												
Air	68	62	74	77	80	83	83	85	83	79	76	69
Sewage	80	77	77	81	80	83	82	82	83	83	84	78

---

[a] Raw sewage flow was split between the original and new plants beginning in September 1980.

[b] Million gallons per day, equivalent to 3.78×10^6 liters/day.

TABLE 32. INFLUENT (RAW SEWAGE) WATER QUALITY FOR THE VIRGINIA KEY SEWAGE TREATMENT PLANT DURING 1980 (mg/liter unless otherwise indicated)

	Month											
	J	F	M	A	M	J	J	A	S	O	N	D
pH (units)	7.1	7.1	7.2	7.1	7.1	7.0	6.9	7.1	7.1	7.2	7.2	7.2
Alkalinity	210	210	210	220	240	230	200	210	200	200	210	210
Chloride	840	820	730	740	830	820	660	680	760	690	720	740
Salinity (ppt)[a]	1.6	1.5	1.4	1.4	1.5	1.5	1.2	1.3	1.4	1.3	1.3	1.4
BOD	132	124	145	117	123	113	101	102	99	105	126	110
Total Residue	2240	2110	1950	1990	2180	2130	1670	1720	1830	1720	1830	1810
Total Fixed Residue	1540	1500	1310	1380	1520	1490	1190	1200	1300	1250	1330	1320
Total Non-filterable Residue	-	174	194	177	178	174	155	136	124	-	117	141

---

[a] Salinity (parts-per-thousand) calculated using the following equation:

$$S\ (\text{ppt}) = 0.030 + 1.8050\ \text{Cl}\ (\text{ppt}).$$

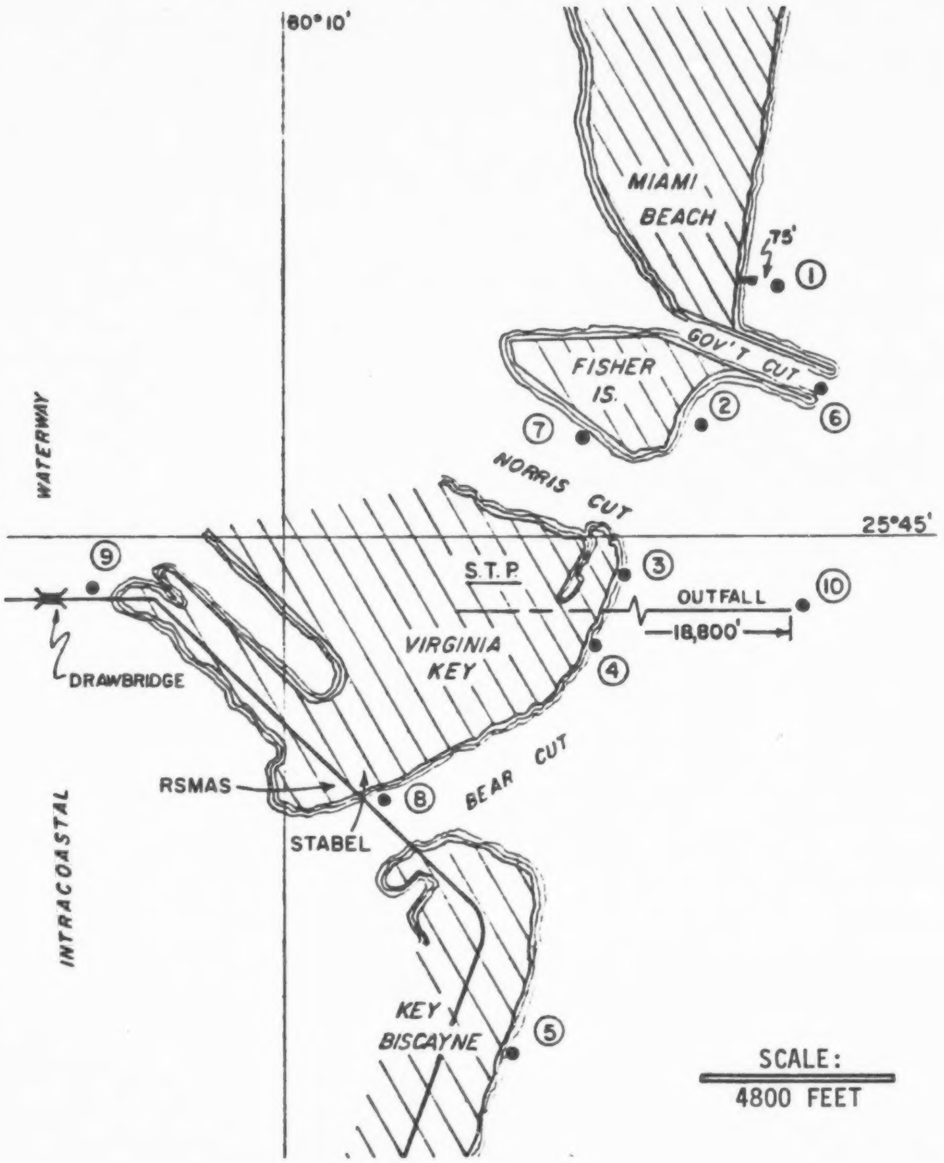
TABLE 33. FINAL (BIOLOGICAL SECONDARY TREATED) EFFLUENT WATER QUALITY FOR THE VIRGINIA KEY SEWAGE TREATMENT PLANT DURING 1980 (mg/liter unless otherwise indicated)

	Month											
	J	F	M	A	M	J	J	A	S	O	N	D
pH (units)	7.1	7.2	7.2	7.1	7.1	7.1	7.1	7.1	7.1	7.3	7.3	7.2
Chloride	830	810	710	730	810	810	670	650	750	660	700	750
Salinity (ppt)[a]	1.5	1.5	1.3	1.3	1.5	1.5	1.2	1.2	1.4	1.2	1.3	1.4
BOD	42	42	51	49	59	59	30	39	25	23	33	21
Total Residue	2170	1970	1810	1900	2030	2030	1700	1610	1770	1610	1740	1720
Total Non-filterable Residue	-	36	50	54	60	65	34	56	31	-	31	31
COD	85	55	110	140	145	175	124	140	125	88	98	-
NITROGEN												
Organic	7.0	7.0	7.6	6.8	7.9	11.8	8.4	11.6	9.4	8.8	9.7	-
Ammonia	12.4	11.2	14.3	12.0	12.9	14.8	11.4	13.4	14.5	10.7	10.8	-
Nitrite	0.3	0.8	0.3	0.2	0.8	0.5	0.5	0.3	0.4	0.3	0.4	-
PHOSPHATE												
Total	4.8	4.1	6.1	6.0	5.7	6.8	4.1	4.4	3.9	2.8	9.0	-
Ortho	3.0	4.6	3.9	4.3	4.0	4.8	3.0	3.8	3.2	2.0	8.0	-

---

[a] Salinity (parts-per-thousand) calculated using the following equation:

$$S(ppt) = 0.030 + 1.8050\ Cl\ (ppt).$$


80° 10'
MIAMI BEACH
75'
①
GOV'T CUT
FISHER IS.
②
⑥
⑦
NORRIS CUT
25°45'
WATERWAY
⑨
S.T.P.
③
OUTFALL
⑩
18,800'
VIRGINIA KEY
④
DRAWBRIDGE
RSMAS
⑧
BEAR CUT
STABEL
INTRACOASTAL
KEY BISCAYNE
⑤
SCALE:
4800 FEET

OFFSHORE AND BAY SAMPLING

Figure 13. Surface seawater sampling stations for coliform bacteria off the Virginia Key Sewage Treatment Plant (S.T.P.) submarine outfall. (Map courtesy of Metro-Dade Water and Sewer Authority.)

TABLE 34. AVERAGE MONTHLY CONCENTRATIONS OF COLIFORM BACTERIA (10^3 M.P.N.) IN FINAL (BIOLOGICAL SECONDARY TREATED) EFFLUENT AND SURFACE SEAWATER AROUND THE VIRGINIA KEY SEWAGE TREATMENT PLANT, 1980

Sampling Site		Month											
		J	F	M	A	M	J	J	A	S	O	N	D
Effluent	Total	10,600	29,900	16,000	14,800	16,300	11,200	59,300	27,500	14,200	28,700	11,700	6,800
	Fecal	2,800	10,800	4,300	2,300	5,300	3,200	6,100	4,000	1,700	7,400	3,200	1,800
#1	Total	3	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
	Fecal	3	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
#2	Total	3	<0.2	<0.2	0.9	<0.2	<0.2	0.3	<0.2	<0.2	<0.2	<0.2	<0.2
	Fecal	0.3	<0.2	<0.2	<0.1	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
#3	Total	30	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	1.3	<0.2	<0.2	<0.2
	Fecal	0.3	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	0.4	<0.2	<0.2	<0.2
#4	Total	3	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
	Fecal	0.3	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
#5	Total	1	<0.2	0.3	<0.1	<0.2	<0.1	<0.2	<0.2	0.4	<0.2	<0.2	<0.2
	Fecal	0.4	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
#6	Total	7	<0.2	<0.2	0.6	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
	Fecal	7	<0.2	<0.2	0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
#7	Total	7	<0.2	<0.2	0.5	<0.2	<0.2	<0.2	<0.2	3.1	2.7	0.2	<0.2
	Fecal	2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	1.5	0.9	<0.2	<0.2
#8	Total	3	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	0.2	<0.2
	Fecal	3	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
#9	Total	0.3	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	27	<0.2	<0.2
	Fecal	0.3	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	27	<0.2	<0.2
#10	Total	330	3,500	11,900	410	160	120	170	280	170	210	250	220
	Fecal	180	590	2,900	100	35	35	27	90	95	95	38	57

TABLE 35. TRACE METAL CONCENTRATIONS (ug/liter) IN INFLUENT (RAW SEWAGE) AND FINAL (BIOLOGICAL SECONDARY TREATED) EFFLUENT AT THE VIRGINIA KEY SEWAGE TREATMENT PLANT, 24 MARCH 1977

	Cd	Cr	Cu	Fe	Mn	Pb	Zn
Influent	8.88	1.86	32.3	850.9	3.5	54.2	177.8
Effluent	1.72	7.58	13.0	427.9	3.6	5.6	42.5

TABLE 36. TRACE METAL CONCENTRATIONS (ug/liter) IN INFLUENT (RAW SEWAGE) AND FINAL (BIOLOGICAL SECONDARY TREATED) EFFLUENT AT THE VIRGINIA KEY SEWAGE TREATMENT PLANT, JULY AND SEPTEMBER, 1977[a]

	(A) July 26-27							(B) July 29-30							(C) September 14						
	0600	1000	1400	1800	2200	0200	0600	0600	1000	1400	1800	2200	0200	0600	1128	1300	1400	1500	1624	1724	1724
				Influent							Influent				Influent			Effluent			
Cd	ND[b]	5.4	7.3	3.8	2.3	3.8	TD[c]	TD	ND	ND	ND	TD	ND	ND	28.5	28.0	34.0	9.4	10.7	10.8	8.7
Cu	ND	38.0	107.0	69.0	26.0	20.0	12.0	18.0	ND	ND	52.0	16.0	ND	ND	29.0	32.0	56.0	24.0	44.0	30.0	22.0
Fe	ND	290	415	800	245	255	TD	10	ND	ND	45	25	ND	ND	475	265	430	250	210	185	230
Pb	ND	20.5	17.0	37.0	16.5	5.7	2.5	TD	ND	ND	TD	TD	ND	ND	19.0	20.0	30.0	2.5	5.5	7.8	5.7
Zn	ND	50	216	50	42	24	TD	ND	ND	ND	ND	TD	ND	ND	216	42	216	9	20	26	18

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[a] Tables A and B represent two 24-hour diurnals of the untreated (raw) sewage entering the plant. Table C presents analysis of untreated and treated sewage from the same water parcel. Water parcel was treated using resident time calculations.

[b] Not detected.

[c] Trace detected.

TABLE 37. CONCENTRATIONS (mg/liter)[a] OF GENERAL WATER QUALITY CONSTITUENTS IN UNTREATED (RAW SEWAGE) WASTEWATER AT THE STABEL FACILITY, 1980

Date	Total Residue	Total Volatile Residue	Total Non-filterable Residue	Volatile Nonfilterable Residue	Turbidity (NTU)	pH (units)	B.O.D.	Organic Carbon
March 10-April 2	1390	390	260	NA	35	7.1	130	83
May 7	NA[b]	NA	NA	NA	NA	NA	NA	NA
May 9	NA	NA	NA	NA	NA	7.9	130	85
May 10	NA	NA	NA	NA	42	8.1	73	NA
May 13	NA	NA	NA	NA	58	7.8	110	NA
May 15	1100	310	13	6	35	7.6	130	72
May 17	1910	360	260	170	63	8.4	150	100
May 19	1180	300	170	56	40	7.1	120	93
June 9	NA	NA	NA	NA	NA	7.9	130	NA
June 11	1560	NA	200	80	37	8.0	120	86
Sept.22	1690	310	120	22	33	NA	NA	NA
Sept.30	1360	280	120	16	33	NA	180	81
Oct. 2	1130	380	120	21	34	6.8	160	88
Median	1380	310	170	22	36	7.8	130	86
Ave.	1420	330	160	53	41	7.7	130	86
S.E.	100	20	30	22	3	0.2	8	3

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[a] Unless otherwise indicated.

[b] Not analyzed.

TABLE 38. CONCENTRATIONS (mg/liter)[a] OF GENERAL WATER QUALITY CONSTITUENTS IN PRIMARY TREATED WASTEWATER AT THE STABEL FACILITY, 1980

Date	Total Residue	Total Volatile Residue	Total Non-filterable Residue	Volatile Nonfilterable Residue	Turbidity (NTU)	pH (units)	B.O.D.	Organic Carbon
March 10-April 2	1140	300	160	NA	28	7.2	90	69
May 7	1640	410	90	58	18	NA	NA	NA
May 9	NA	NA	NA	NA	NA	NA	NA	NA
May 10	NA	NA	NA	NA	43	7.9	56	NA
May 13	NA	NA	NA	NA	NA	7.8	63	NA
May 15	1070	320	92	48	36	7.8	76	66
May 17	1600	190	130	60	24	8.0	54	64
May 19	820	330	94	40	44	7.7	88	68
June 9	1880	310	680	160	54	7.8	80	74
June 11	1660	NA	150	76	23	7.5	74	72
Sept.22	2110	380	120	26	30	NA	NA	NA
Sept.30	1540	270	61	14	23	NA	96	66
Oct. 2	1104	210	59	12	28	6.5	120	70
Median	1570	310	110	48	28	7.8	78	68
Ave.	1460	300	160	55	32	7.6	80	69
S.E.	130	20	60	15	3	0.2	6	1

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[a] Unless otherwise indicated.

TABLE 39. CONCENTRATIONS (mg/liter)[a] OF GENERAL WATER QUALITY CONSTITUENTS IN CHEMICAL FLOCCULANT TREATED WASTEWATER AT THE STABEL FACILITY, 1980

Date	Total Residue	Total Volatile Residue	Total Non-filterable Residue	Volatile Nonfilterable Residue	Turbidity (NTU)	pH (units)	B.O.D.	Organic Carbon
March 10-April 2	1080	190	43	NA	15	6.9	36	39
May 7	1290	230	59	23	18	NA	NA	NA
May 9	1620	270	50	27	11	7.3	39	29
May 10	NA	NA	NA	NA	NA	NA	NA	NA
May 13	1800	260	72	NA	12	NA	16	41
May 15	1660	310	NA	NA	20	8.0	30	36
May 17	1510	210	96	40	24	8.0	28	29
May 19	NA	NA	NA	NA	17	6.7	20	26
June 9	NA	NA	NA	NA	NA	NA	NA	NA
June 11	1820	300	136	16	16	7.9	46	44
Sept.22	NA	NA	NA	NA	NA	NA	NA	NA
Sept.30	NA	NA	NA	NA	NA	NA	NA	NA
Oct. 2	NA	NA	NA	NA	NA	NA	NA	NA
Median	1620	260	54	25	16	7.1	30	36
Ave.	1540	250	76	26	17	7.5	31	35
S.E.	100	20	14	5	1	0.2	4	3

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[a] Unless otherwise indicated.

TABLE 40. CONCENTRATIONS (mg/liter)[a] OF GENERAL WASTEWATER CONSTITUENTS IN BIOLOGICAL SECONDARY TREATED WASTEWATER AT THE STABEL FACILITY, 1980

Date	Total Residue	Total Volatile Residue	Total Non-filterable Residue	Volatile Nonfilterable Residue	Turbidity (NTU)	pH (units)	B.O.D.	Organic Carbon
March 10-April 2	1250	190	30	NA	5	7.2	34	20
May 7	1350	300	36	17	18	NA	NA	NA
May 9	NA	NA	NA	NA	NA	NA	NA	NA
May 10	NA	NA	NA	NA	32	7.8	2	NA
May 13	NA	NA	NA	NA	5	NA	NA	NA
May 15	1490	260	96	54	8	6.7	7	26
May 17	2100	270	54	21	10	7.9	10	21
May 19	1720	370	37	23	9	7.1	10	16
June 9	2020	280	24	8	8	6.9	6	16
June 11	NA	NA	NA	NA	NA	NA	NA	NA
Sept.22	1510	190	6	2	3	NA	NA	NA
Sept.30	2040	390	18	2	4	NA	16	14
Oct. 2	1500	290	140	24	42	6.8	46	62
Median	1510	280	36	19	8	7.1	10	20
Ave.	1660	280	49	19	13	7.2	16	25
S.E.	110	20	14	6	4	0.2	5	6

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[a] Unless otherwise indicated.

Treatment Level	TSS (mg/l)	Turbidity (NTU)	BOD (mg/l)	TOC (mg/l)
Raw	170	36	130	86
Primary	110	28	78	68
Chem. Floc.	54	16	30	36
Secondary	36	8	10	21

Tables 41-44 list the concentrations of the nitrogen and phosphorus nutrients monitored in the four STABEL wastewaters during 1980. For the non-chlorinated wastewaters, with increasing treatment level successive decreases were observed for median concentrations of ammonia, while the opposite was true for nitrate:

Treatment Level	NH_3-N (mg/l)	NO_3-N (mg/l)	Sum N (mg/l)
Raw	17	0.7	18
Primary	16	2.4	18
Chem. Floc.	14	5.6	20
Bio.Secondary	11	9.3	20

The level of nitrite nitrogen in all the non-chlorinated wastewaters (approximately 0.1 mg/liter) was two orders of magnitude below that for ammonia nitrogen, and the sum of ammonia nitrogen and nitrate nitrogen (18-20 mg/liter) did not change significantly with treatment. This also was true for the median concentrations of orthophosphate (0.4-0.6 mg/liter). Chlorination had no clear discernable effect on the concentrations of measurable ammonia in the STABEL wastewaters. Median values for the non-chlorinated vs. chlorinated samples of the primary and chemical flocculant wastewaters were 16 vs. 14 and 14 vs. 14 mg/liter, respectively. For the biological secondary wastewater samples, median concentrations of ammonia nitrogen in the nonchlorinated and chlorinated samples were 11 and 4.0 mg/liter, respectively. However, these differences, as determined by the nonparametric two-tailed rank sum test (Tate and Clelland, 1957) were not significantly different ($p>1.10$).

Median concentrations of the five potentially toxic trace metals cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), and zinc (Zn) generally ranged from 1-100 parts-per-billion (ug/liter) in the unfiltered wastewater samples (Tables 45-48), and were consistent with the values reported for the Virginia Key STP (Tables 35 and 36). The STABEL values always were lower in the chemical flocculant and biological secondary wastewaters than they were in the raw and primary wastewaters. For copper and zinc, order-of-magnitude decreases were observed.

Concentrations of the trace synthetic organic compounds quantified in the STABEL wastewater samples are listed in

TABLE 41. CONCENTRATIONS (mg/liter) OF NUTRIENTS IN INFLUENT (RAW SEWAGE) WASTEWATER AT THE STABEL FACILITY, 1980

Date	NH_4-N	NO_2-N	NO_3-N	PO_4-P
April 17	17	0.1	0.2	0.4
May 5	16	0.1	0.3	0.4
May 7	17	0.0	0.2	0.3
May 9	19	0.0	0.2	0.3
May 11	18	0.1	45	1.9
May 13	13	0.1	18	1.6
May 15	14	0.1	13	2.7
May 17	11	0.2	0.7	0.5
May 19	11	0.1	7.6	2.5
June 9	17	NA	NA	NA
June 11	17	NA	NA	NA
Median	17	0.1	0.7	0.5
Ave.	16	0.1	9.5	1.2
S.E.	1	0.0	5.0	0.3

TABLE 42. CONCENTRATIONS (mg/liter) OF NUTRIENTS IN NON-CHLORINATED AND CHLORINATED PRIMARY TREATED WASTEWATER AT THE STABEL FACILITY, 1980

Date	NH_4-N		NO_2-N		NO_3-N		PO_4-P	
	Non-Chlor.	Chlor.	Non-Chlor.	Chlor.	Non-Chlor.	Chlor.	Non-Chlor.	Chlor.
April 17	19	NA	0.1	NA	2.0	NA	0.3	NA
May 5	15	13	0.1	0.1	0.2	0.1	0.2	0.3
May 7	17	17	0.1	0.1	13	0.2	0.4	0.3
May 9	19	19	0.0	0.1	0.2	0.2	0.4	0.4
May 11	17	12	0.2	0.2	12	1.4	1.4	2.6
May 13	14	14	0.1	0.1	2.7	2.7	1.6	1.6
May 15	15	8.3	0.1	0.1	1.4	0.8	2.6	0.4
May 17	8.5	7.7	0.6	1.9	28	4.1	0.8	0.9
May 19	13	0.7	0.2	0.1	NA	7.2	2.9	2.7
June 9	17	15	NA	NA	NA	NA	NA	NA
June 11	16	17	NA	NA	NA	NA	NA	NA
Median	16	14	0.1	0.1	2.4	1.1	0.8	0.6
Ave.	16	12	0.2	0.3	7.4	2.1	1.2	1.2
S.E.	1	2	0.1	0.2	3.5	0.5	0.3	0.4

TABLE 43. CONCENTRATIONS (mg/liter) OF NUTRIENTS IN NON-CHLORINATED AND CHLORINATED CHEMICAL FLOCCULANT TREATED WASTEWATER AT THE STABEL FACILITY, 1980

Date	NH_4-N		NO_2-N		NO_3-N		PO_4-P	
	Non-Chlor.	Chlor	Non-Chlor.	Chlor.	Non-Chlor.	Chlor.	Non-Chlor.	Chlor.
April 17	18	NA	0.1	NA	28	NA	0.4	NA
May 5	15	17	0.1	0.1	26	1.5	0.3	0.3
May 7	19	18	0.1	0.1	11	3.9	0.3	0.4
May 9	17	15	0.1	1.2	6.4	13	0.4	0.8
May 11	14	13	0.3	0.3	NA	12	0.5	0.4
May 13	18	28	0.1	2.0	3.6	NA	0.9	1.9
May 15	13	6.6	0.1	0.1	4.9	10	0.4	0.4
May 17	<1	0.5	0.5	0.5	2.2	3.7	0.4	0.3
May 19	10	9.4	0.5	1.2	1.4	13	0.4	0.4
June 9	13	13	NA	NA	NA	NA	NA	NA
June 11	13	15	NA	NA	NA	NA	NA	NA
Median	14	14	0.1	0.4	5.6	10	0.4	0.4
Ave.	14	14	0.2	0.7	10	8.2	0.4	0.6
S.E.	2	2	0.1	0.3	4	1.9	0.1	0.2

TABLE 44. CONCENTRATIONS (mg/liter) OF NUTRIENTS IN NON-CHLORINATED AND CHLORINATED BIOLOGICAL SECONDARY TREATED WASTEWATER AT THE STABEL FACILITY, 1980

Date	NH_4-N		NO_2-N		NO_3-N		PO_4-P	
	Non-Chlor.	Chlor.	Non-Chlor.	Chlor.	Non-Chlor.	Chlor.	Non-Chlor.	Chlor.
April 17	11	NA	0.1	NA	0.3	NA	0.3	NA
May 5	15	11	0.1	0.1	0.2	0.2	0.2	0.2
May 7	19	13	0.1	0.1	0.2	0.2	0.4	0.2
May 9	16	13	0.1	0.1	22	35	0.2	0.7
May 11	11	11	0.5	0.2	1.4	1.7	0.8	1.6
May 13	12	5.3	2.0	1.2	18	21	1.9	2.3
May 15	2.8	0.4	14	7.4	9.3	3.7	2.3	1.2
May 17	<1	1	0.9	0.3	12	6.1	0.5	1.6
May 19	2.5	2.8	0.1	1.9	27	150	2.3	1.6
June 9	6.7	1.3	NA	NA	NA	NA	NA	NA
June 11	6.7	<0.1	NA	NA	NA	NA	NA	NA
Median	11	4.0	0.1	0.2	9.3	2.7	0.5	1.4
Ave.	9.4	5.9	2.0	1.4	10	27	1.0	1.2
S.E.	1.8	1.7	1.5	0.9	3	19	0.3	0.3

TABLE 45. CONCENTRATIONS (ug/liter) OF TRACE METALS IN INFLUENT (RAW SEWAGE) WASTEWATER AT THE STABEL FACILITY, 1980

Date[a]	Cd	Cr	Cu	Pb	Zn
Dec. 24-28	4	11	5	24	120
Mar. 16-22 (R1)[b]	8	39	31	20	132
Mar. 16-22 (R2)[b]	8	24	36	17	220
Apr. 6- 8	6	22	28	17	140
Apr. 9-12	<2	29	43	28	140
Apr. 14-20	<2	61	32	26	160
May 2-10	9	38	28	9	80
Median	6	29	31	20	140
Average	5.6	32	29	20	140
S.E.	1.1	6	4	2	16

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[a] Compositing period (December composite in 1979).

[b] Replicate composite samples.

TABLE 46. CONCENTRATIONS (ug/liter) OF TRACE METALS IN PRIMARY TREATED WASTEWATER AT THE STABEL FACILITY, 1980

Date[a]	Cd	Cr	Cu	Pb	Zn
Dec. 24-28	2	7	31	19	96
Mar. 16-22	11	61	38	42	250
Apr. 6- 8 (R1)[b]	4	9	18	9	83
Apr. 6- 8 (R2)[b]	4	6	13	9	100
Apr. 9-12	<2	26	48	16	89
Apr. 14-20	<2	42	38	15	130
May 2-10	<2	44	80	20	160
Median	2	44	38	16	100
Average	3.9	28	38	19	130
S.E.	1.2	8	8	4	22

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[a] Compositing period (December composite in 1979).

[b] Replicate composite samples.

TABLE 47. CONCENTRATIONS (ug/liter) OF TRACE METALS IN CHEMICAL FLOCCULANT TREATED WASTEWATER AT THE STABEL FACILITY, 1980

Date[a]	Cd	Cr	Cu	Pb	Zn
Dec. 24-28	<2	3	8	6	30
Mar. 16-22	<2	17	5	17	18
Apr. 6- 8	<2	4	3	7	16
Apr. 9-12	<2	12	<2	ND[b]	11
Apr. 14-20	<2	24	26	12	63
May 2-10	<2	13	3	ND	ND
Median	<2	12	3	10	28
Average	<2	12	7.8	10	28
S.E.	--	3	3.7	2	9

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[a] Compositing period (December composite in 1979).

[b] Not detected.

TABLE 48. CONCENTRATIONS (ug/liter) OF TRACE METALS IN BIOLOGICAL SECONDARY TREATED WASTEWATER AT THE STABEL FACILITY, 1980

Date[a]	Cd	Cr	Cu	Pb	Zn
Dec. 24-28	<2	ND[b]	3	4	25
Mar. 16-22	<2	<2	6	4	NA[c]
Mar. 23-29	<2	ND	<2	<2	11
Apr. 6- 8	<2	4	3	7	16
Apr. 9-12	<2	14	3	ND	3
Apr. 14-20	46[d]	32	32	8	79
May 2-10	<2	17	4	ND	27
Median	<2	14	3	4	20
Average	<2	14	7.6	5.0	27
S.E.	--	5	4.1	1.1	11

---

[a] Compositing period (December composite in 1979).

[b] Not detected.

[c] Not analyzed.

[d] Value dropped.

Tables 49-52. Total levels of the chlorinated pesticides generally did not exceed about 1 part-per-billion (ug/liter), while the measurable polychlorinated biphenyls (PCB) values generally were below about 4 ppb. Considerably higher concentrations (roughly 100-200 ppb) of total phthalic acid esters (PAE) were observed; the following PAEs usually were quantified in the STABEL wastewaters:

Di-isobutyl phthalate (DIBP)
Di-butyl phthalate (DBP)
Butylglycolbutyl phthalate (BGBP)
Butylbenzyl phthalate (BBP)
Di-ethylhexyl phthalate (DEHP)

No significant changes in wastewater concentration with treatment were observed for any of these trace synthetic organics measured in the STABEL samples.

Comprehensive analyses of the settled seawater, used to dilute the STABEL wastewaters for the bioeffects exposure tests, were not conducted. Therefore, estimated levels of the target nutrients, trace metals, and synthetic organics in this seawater (listed below) were obtained from various sources, so that the concentrations of these constituents in the various exposure tanks can be estimated.

NH_3-N: 0.04
NO_2-N: <0.01
NO_3-N: 0.08
PO_4-P: <0.01

Cd: 0.02
Cr: 0.2[a]
Cu: 0.4
Pb: 0.4
ZN: 4

Chl. Pest.: <0.01[b]
PCB : <0.01[b]
PAE : 1[c]

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[a] Nearshore southern California (Jan and Young, 1978).
[b] Nearshore southern California (Young, 1982).
[c] Gulf of Mexico (Corcoran and Curry, 1978).

TABLE 49. CONCENTRATIONS (ug/liter) OF SYNTHETIC ORGANICS IN INFLUENT (RAW SEWAGE) WASTEWATER AT THE STABEL FACILITY, 1980

Date[a]	Total. Chlor. Pesticides	Polychlorinated Biphenyls			Phthalic Acid Esters					
		1016	1254	Total	DIBP	DBP	BGBP	BBP	DEHP	TOTAL
Dec. 24-28	0.87	0.08	0.45	0.53	44	19	18	13	6	100
Mar. 15-20	0.46	0.04	0.23	0.27	39	17	21	15	11	100
Apr. 6- 8	0.08	1.2	2.7	3.9	2.4	6.0	4.5	14	9.1	36
May 9-10	NA[b]	NA	NA	NA	3.5	4.9	7.8	9.1	9.2	34
May 13-14	0.80	1.0	2.0	3.0	4.1	9.7	68	ND[c]	11	93
Median	0.63	0.90	1.2	1.8	4.1	9.7	18	14.6	2	93
Average	0.55	0.58	1.3	1.9	19	11	24	13	9.3	73
S.E.	0.18	0.30	0.6	0.9	9	3	11	1	0.9	15

---

[a] Compositing period (December composite in 1979).

[b] Not analyzed.

[c] Not detected.

TABLE 50. CONCENTRATIONS (ug/liter) OF SYNTHETIC ORGANICS IN PRIMARY TREATED WASTEWATER AT THE STABEL FACILITY, 1980

Date[a]	Total. Chlor. Pesticides	Polychlorinated Biphenyls			Phthalic Acid Esters					
		1016	1254	Total	DIBP	DBP	BGBP	BBP	DEHP	TOTAL
Mar. 8-15	2.1	1.8	28	30	24	9.6	7.6	6.0	6.2	53
Mar. 16-22	0.64	0.09	0.07	0.16	5.4	22	29	19	16	91
Mar. 23-29	0.05	0.55	1.5	2.0	18	21	39	36	26	140
Apr. 9-12	0.15	0.01	0.06	0.07	2.7	2.7	52	51	125	230
Apr. 14-20	NA[b]	NA	NA	NA	4.6	7.1	ND[c]	8.6	11	31
May 13-14	NA	NA	NA	NA	10	11	8.8	19	19	69
Median	0.40	0.32	0.78	1.1	7.7	10	29	19	18	93
Average	0.74	0.61	7.4	8.1	11	12	27	23	34	100
S.E.	0.47	0.41	6.9	7.3	4	3	9	7	18	30

---

[a] Compositing period.

[b] Not analyzed.

[c] Not detected.

TABLE 51. CONCENTRATIONS (ug/liter) OF SYNTHETIC ORGANICS IN CHEMICAL FLOCCULANT TREATED WASTEWATER AT THE STABEL FACILITY, 1980

Date[a]	Total. Chlor. Pesticides	Polychlorinated Biphenyls			Phthalic Acid Esters					
		1016	1254	Total	DIBP	DBP	BGBP	BBP	DEHP	TOTAL[c]
Dec. 24-28	5.8	0.03	0.18	0.21	91	36	44	24	14	210
Mar. 16-20	1.2	0.23	0.76	0.99	ND[b]	ND	ND	11	13	96
Mar. 23-29	1.2	0.12	0.32	0.44	80	34	ND	28	37	180
Median	1.2	0.12	0.32	0.44	86	35	44	24	14	180
Average	2.7	0.13	0.42	0.53	86	35	44	21	21	160
S.E.	1.5	0.06	0.17	0.24	1	1	0	6	10	34

---

[a] Compositing period (December composite in 1979).

[b] Not detected.

[c] Includes unidentified PAEs.

TABLE 52. CONCENTRATIONS (ug/liter) OF SYNTHETIC ORGANICS IN BIOLOGICAL SECONDARY TREATED WASTEWATER AT THE STABEL FACILITY, 1980

		Polychlorinated Biphenyls			Phthalic Acid Esters					
Date[a]	Total. Chlor. Pesticides	1016	1254	Total	DIBP	DBP	BGBP	BBP	DEHP	TOTAL
Dec. 24-28	NA[b]	NA	NA	NA	54	23	ND[c]	18	12	110
Apr. 9-11	1.1	3.6	0.9	4.5	4.4	4.6	11	25	110	160
May 5-10	NA	NA	NA	NA	10	3.5	2.5	22	23	61
Median	1.1	3.6	0.9	4.5	10	4.6	6.8	22	23	110
Average	1.1	3.6	0.9	4.5	23	10	6.8	22	48	110
S.E.	--	--	--	--	19	8	4.3	2	38	29

---

[a] Compositing period (December composite in 1979).

[b] Not analyzed.

[c] Not detected.

SECTION 9

STABEL EXPERIMENTS WITH FISH AND SHRIMP

INTRODUCTION

This and the following section present all of the results obtained from the diluted wastewater exposure experiments conducted to date at STABEL. These experiments were designed as preliminary tests to provide information on characteristics of the dosing media and associated biological responses, which could be used to optimize the long-term (>100 days) exposures using indigenous marine organisms for which STABEL was intended.

PROCEDURES

Five bioeffects experiments using southeast Florida fish and shrimp were initiated in the STABEL facility, generally following the guidelines specified by the SEFOOS-STABEL Operational Procedures manual (Appendix C). Details of the experiments are presented in Table 53.

Average water temperatures measured in the exposure tanks for December, March, and June were approximately 19°C, 21°C, and 27°C, respectively; even through different tanks were shaded at different times of the day, temperatures rarely varied more than 1°C from tank to tank because of the flowing water supply. Throughout the experimental period, the salinity of the exposure water ranged from 33 to 36 $^{o}/oo$, and varied by no more than 1 $^{o}/oo$ per day in the 1:30 dilution tanks. Levels of dissolved oxygen were monitored with a YSI oxygen meter standarized against the Winkler method. On only one day did the DO level fall below saturation (and then only to 95 percent saturation). On all other days, the DO was saturated or super-saturated, sometimes to 115 to 120 percent (this is a common phenomenon in seawater). As expected, the pH remained between 8.0 to 8.1 regardless of treatment.

The specimens to be tested were collected from uncontaminated regions in or near the Florida Keys, and acclimated in the large concrete flowing seawater holding tanks in the STABEL facility for at least one week before initiation of an experiment. Generally, an experiment was begun with 20 individuals per tank, and 3 replicate tanks per treatment and dilution were used. (In Experiment II, the initial number of specimens per tank ranged from 24 to 37.)

TABLE 53. FISH AND SHRIMP SPECIES TESTED IN STABEL BIOEFFECTS EXPERIMENTS

Experiment Number	Observation Period	Common Name	Scientific Name	Ave. Wet Weight (g)	Ave. Std. Length (mm)
I	12/20/79 - 1/31/80	Silver Jenny	*Eucinostomus gula*	1.82	36.8
II	12/20/79 - 1/31/80	Hardhead Silverside	*Atherinomorus stipes*	0.75	37.2
III	3/7/80 - 4/10/80	Sheepshead Minnow	*Cyprinodon variegatus*	4.01	41.1
IV	4/16/80 - 5/22/80	Pink Shrimp	*Penaeus duorarum*	2.50	6.6[a]
V	6/10/80 - 8/3/80	High Hat	*Equetus acuminatus*	DNL[b]	30

---

[a] Rostrum to telson; ave. carapace length: 1.6 cm.

[b] Data not located.

Each tank was checked daily to count and remove dead or moribund individuals. At the end of the experiment, surviving specimens were preserved or frozen. Thirty-one such specimens of *Cyprinodon* collected at the end of Experiment III were submitted for histopathology to Dr. Richard Wolke, Department of Aquaculture Science and Pathology, University of Rhode Island at Kingston, Rhode Island. In addition, pooled samples (usually containing five whole specimens) of *Cyprinodon* and shrimp collected at the end of Experiments III and IV, respectively, were analyzed for toxic trace metals and chlorinated hydrocarbons at RSMAS.

In the following section, the records of daily mortalities observed for the various exposures (treatments and dilutions) in the five bioeffects experiments on fish or shrimp are presented and discussed.

RESULTS AND DISCUSSION

Experiments I and II

Bioeffects studies I and II were conducted simultaneously on the juvenile silver jenny (*Eucinostomus gula*) and adult hardhead silversides (*Atherinomorus stipes*), beginning December 20, 1979. These tests did not include the effect of chlorination, as the appropriate equipment for such exposure had not yet been installed. The silversides had initial feeding difficulty and high mortality due to transfer. Plant failures resulted in termination of treated sewage to the bioeffects tanks on December 30, 1979. However, the experiments were continued for another month to follow the response of these two test fishes to the initial ten days of exposure. The observations were terminated on January 31, 1980. The results of these two initial experiments are presented in Tables 54-57. Because no documentation of the final surving population was located for the third tank of each exposure triplicate set, the total body count for each Tank No. 3 was corrected for the average body count "efficiency" (Body Count divided by: Initial Pop. minus Final Pop.) calculated for Tank No. 1 and No. 2 to obtain an estimated value of the true mortality for Tank No. 3. Values for percent mortality and the corresponding median day until death for each exposure tank then were obtained.

The data from Experiment I (Tables 54 and 55) show the juvenile silver jenny to be a very hardy bioassay species, with very low mortality observed in any of the ten triplicate exposure tests. On the average, more than 95 percent of the specimens survived the initial ten-day exposure to the diluted wastewaters and the subsequent month-long observation period.

In contrast, the adult hardhead silversides appeared to be an overly sensitive species (Tables 56 and 57). The high

TABLE 54. STABEL EXPERIMENT I: OBSERVED MORTALITY VS. TIME FOR *Eucinostomus gula* EXPOSED TO CONTROL SEAWATER OR NON-CHLORINATED WASTEWATER TREATED BY THE PRIMARY SETTLING PROCESS

Treatment	Control Seawater			Primary Settling[a]								
Dilution		-		30:1			100:1			300:1		
Tank No.	1	2	3	1	2	3	1	2	3	1	2	3
Initial Pop.	20	20	20	20	20	20	20	20	20	20	20	20
Final Pop.	20[b]	21[b]	20[c]	20	19	20[c]	19[b]	21[b]	20[c]	20	20	19[c]
% Mortality	0	0	0	0	5	0	10	0	0	0	0	5
Day 1												
2												
3												
4												
5												
6												
7												
8												
9												
10												
11												
12												
13												
14												
15												
16												
17												
18												
19												
20												
21												
22												
23	1						1					
24												
25							1					
26												
27												
28												
29												
30												
31												
32												
33												
34												
35												
36					1							1
37												
38												
39												
40												
41												
42												
Total Count	1	0	0	0	1	0	2	0	0	0	0	1
Median Day	23	-	-	-	36	-	24	-	-	-	-	36

---

[a] Wastewater exposure through Day 10 only.

[b] The reported values for initial population, final population, and/or observed mortality recorded on the laboratory data sheets are not consistent.

[c] Estimated from total body count corrected by average body count efficiency for Tanks 1 and 2.

TABLE 55. STABEL EXPERIMENT I: OBSERVED MORTALITY VS. TIME FOR *Eucinostomus gula* EXPOSED TO NON-CHLORINATED WASTEWATERS TREATED BY THE CHEMICAL FLOCCULATION OR BIOLOGICAL SECONDARY PROCESS

Treatment	Chemical Flocculant[a]									Biological Secondary[a]								
Dilution	30:1			100:1			300:1			30:1			100:1			300:1		
Tank No.	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Initial Pop.	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
Final Pop.	19	20	20[b]	20	20	20[b]	20	20	20[b]	20	19	20[b]	18	18	20[b]	19	18	19[b]
% Mortality	5	0	0	0	0	0	0	0	0	0	5	0	10	10	0	5	10	5
Day 1																		
2																		
3																		
4																		
5																		
6																		
7																		
8																		
9																		
10																		
11																		
12																		
13																		
14																		
15																		
16																1		
17																		
18																		
19																		
20																		
21																		
22																		
23													2					1
24																		
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32																		
33																		
34																		
35																		
36																		
37																		
38																		
39																		
40																		
41																		
42																		
Total Count	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	1	0	1
Median Day	-	-	-	-	-	-	-	-	-	-	-	-	23	-	-	16	-	23

---

[a] Wastewater exposure through Day 10 only.

[b] Estimated from total body count corrected by average body count efficiency for Tanks 1 and 2.

TABLE 56. STABEL EXPERIMENT II: OBSERVED MORTALITY VS. TIME FOR *Atherinomorus stipes* EXPOSED TO CONTROL SEAWATER OR NON-CHLORINATED WASTEWATER TREATED BY THE PRIMARY SETTLING PROCESS

Treatment	Control Seawater			Primary Settling[a]								
Dilution		-		30:1			100:1			300:1		
Tank No.	1	2	3	1	2	3	1	2	3	1	2	3
Initial Pop.	37	24	32	32	26	35	32	30	28	28	25	27
Final Pop.	6	15	10[b]	26	24	15[b]	20	12	20[b]	15	20	15[b]
% Mortality	76	38	69	19	8	57	38	60	29	46	20	44
Day 1	4	1	1			5	1	1		1		1
2	8	2	4			2				6		1
3	2		4				1	1	1	2	3	1
4		1	1					2	1	1	1	4
5			1					1			1	
6	1		1				1					
7			3			1	1		1			1
8	2	1	1					1	1			
9	1						1					1
10						2	1					1
11	2	1					1		1	1		
12											1	
13			1									
14								1			1	
15			1									
16	1											
17												2
18												1
19				1				1		1		1
20							1					
21		1		1								
22				1						2		
23	1											
24												
25					1							
26												
27								1				
28												
29												
30												
31												
32												
33												
34							1					
35	1											
36								1				
37												
38												
39												
40											1	
41												1
42												
Total Count	23	7	18	3	1	10	9	10	5	14	8	15
Median Day	2	4	4	21	25	2	9	6	7	2	4	7

---

[a] Wastewater exposure through Day 10 only.

[b] Estimated from total body count corrected by average body count efficiency for Tanks 1 and 2.

TABLE 57. STABEL EXPERIMENT II: OBSERVED MORTALITY VS. TIME FOR *Atherinomorus stipes* EXPOSED TO NON-CHLORINATED WASTEWATERS TREATED BY THE CHEMICAL FLOCCULATION OR BIOLOGICAL SECONDARY PROCESS

Treatment	Chemical Flocculation[a]									Biological Secondary[a]								
Dilution	30:1			100:1			300:1			30:1			100:1			300:1		
Tank No.	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Initial Pop.	27	29	27	34	29	34	31	31	31	25	24	26	25	25	24	24	26	36
Final Pop.	13	25	19[b]	18	12	27[b]	18	7	15[b]	14	14	11[b]	9	15	11[b]	12	10	14[b]
% Mortality	52	14	30	47	59	21	42	77	52	44	58	42	64	40	54	50	62	61
Day 1	2		3	4	1		1	2		3	3	3	3	2	1			6
2	6		1	3	2	1		4	1	2	3	3	2	1	2	4	2	3
3				1	4	1	2	3			1	2	1		3		2	3
4							1	5	2		1			3		1	1	
5	1			2		1	2	1					1	1			1	
6	1	1	1					1	1	1	1	2						
7	1	1							1	1	1		1				1	
8		1								1				1				
9										1	1					1		
10							1		1									
11									1									
12							1						1					
13									1					1				
14																		
15									1						1			
16																		
17																		
18																		
19								1							1		1	
20																		
21									2				1		1			
22														1			1	
23	2		2	1											1			
24								1							1			
25																	1	
26																		
27																		
28																	1	
29						1												1
30																		
31																		
32																		
33																		
34																		
35													1					
36																		
37																		
38																		
39																		
40																		
41																		
42																		
Total Count	13	3	7	11	7	4	8	18	11	9	11	10	11	10	11	6	11	13
Median Day	2	7	2	2	3	4	4	4	10	2	2	2	3	4	3	2	5	2

---

[a] Wastewater exposure through Day 10 only.

[b] Estimated from total body count corrected by average body count efficiency for Tanks 1 and 2.

mortality experienced even in the control seawater (averaging more than 50 percent) makes difficult any interpretation of the effects of the ten-day exposure to the diluted wastewaters. However, for a given dilution, a direct relationship was observed between the degree of treatment and the median percent mortality value, as summarized below:

	Median Percent Mortality			
Dilution	Primary	Chem. Floc.	Bio. Second.	Control SW
30:1	19	30	44	--
100:1	38	47	54	--
300:1	44	52	61	--
Infinite	--	--	--	69

This summary also indicates that, for any given treatment, mortality increased with increasing wastewater dilution, control seawater yielding the highest median mortality. Further, the median time to death (21 days) for specimens exposed to the lowest dilution of primary effluent was an order-of-magnitude greater than that (2-4 days) measured for any dilution of the other effluents, or control seawater.

Although quite variable, these results suggest that at least the limited (ten-day) exposure to non-chlorinated STABEL wastewaters increased the survival of the hardhead silversides relative to the control case, and that survival increased as the level of treatment or dilution decreased.

Experiment III

Adult sheepshead minnows (Cyprinodon variegatus) were used for Bioeffects Study III. The test specimens were exposed to both chlorinated and non-chlorinated effluents for 34 days between March 7 and April 10, 1980. The results are presented in Tables 58-61. Excessive residual chlorine levels apparently caused the considerable mortality observed in the fish exposed to 30:1 dilutions of chlorinated wastewaters treated by the chemical flocculation and biological secondary processes (median values of 85 percent and 25 percent) after four to five days of exposure, and to the 30:1 dilutions of chlorinated wastewaters treated by primary settling (100 percent) after 14 days of exposure. Otherwise, only scattered mortality occurred in all other tanks for the duration of the experiment, as is illustrated by the median mortality values for the non-chlorinated effluents summarized below:

TABLE 58. STABEL EXPERIMENT III: OBSERVED MORTALITY VS. TIME FOR Cyprinodon variegatus EXPOSED TO CONTROL SEAWATER

Tank No.	1	2	3	5	6
Initial Pop.	20	20	20	20	20
Final Pop.	20	20	18	19	18
% Mortality	0	0	10	5	10
Day 1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17			1		1
18					
19				1	
20					
21					
22					
23					
24					
25					
26					
27					
28					
29					
30					
31					
32					
33			1		
34					1
Total Count	0	0	2	1	2
Median Day	-	-	25	19	26

TABLE 59. STABEL EXPERIMENT III: OBSERVED MORTALITY VS. TIME FOR *Cyprinodon variegatus* EXPOSED TO WASTEWATER TREATED BY THE PRIMARY SETTLING PROCESS

	Non-Chlorinated									Chlorinated								
Dilution	30:1			100:1			300:1			30:1			100:1			300:1		
Tank No.	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Initial Pop.	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
Final Pop.	20	20	17	20	19	19	19	20	13	0	0	0	20	18	20	20	19	20
% Mortality	0	0	15	0	5	5	5	0	35	100	100	100	0	10	0	0	5	0
Day 1																		
2																		
3						1												
4																		
5																		
6																		
7																		
8																		
9																		
10																		
11										1								
12				1					1									
13			1							1							1	
14[a]				1														
15					1				1	18	20	19						
16			1															
17							1		1									
18																		
19																		
20			1						1									
21																		
22																		
23																		
24																		
25																		
26																		
27			1															
28																		
29																		
30									2									
31									1									
32																		
33																		
34																		
Total Count	0	0	4	2	1	1	1	0	7	20	20	19	0	0	0	0	1	0
Median Day	-	-	18	13	15	13	17	-	20	15	15	15	-	-	-	-	13	-

---

[a] Excessive chlorination recorded.

TABLE 60. STABEL EXPERIMENT III: OBSERVED MORTALITY VS. TIME FOR *Cyprinodon variegatus* EXPOSED TO WASTEWATER TREATED BY THE CHEMICAL FLOCCULATION PROCESS

	Non-Chlorinated									Chlorinated								
Dilution	30:1			100:1			300:1			30:1			100:1			300:1		
Tank No.	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Initial Pop.	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
Final Pop.	17	18	20	17	19	20	19	20	20	8	3	2	16	19	17	19	19	20
% Mortality	15	10	0	15	5	0	5	0	0	60	85	90	20	5	15	5	5	0
Day 1																		
2																		
3																		
4										3		4						
5										1	4	4						
6										8	1	3						
7											1	1	1					
8											11	6	1		2			
9														1				
10																		
11				1														
12																		
13		1																
14	1															1		
15				1	1										1			
16		1																
17	1																	
18																		
19				1														
20																		
21							1											
22																		
23																		
24																		
25																		
26																		
27													1					
28	1												1					
29																		
30																		
31	1																	
32																		
33																		
34																		
Total Count	4	2	0	3	1	0	1	0	1	12	17	18	4	1	3	1	0	0
Median Day	22	14	-	15	15	-	21	-	34	6	8	6	18	9	8	14	-	-

TABLE 61. STABEL EXPERIMENT III: OBSERVED MORTALITY VS. TIME FOR *Cyprinodon variegatus* EXPOSED TO WASTEWATER TREATED BY THE BIOLOGICAL SECONDARY PROCESS

	Non-Chlorinated									Chlorinated								
Dilution	30:1			100:1			300:1			30:1			100:1			300:1		
Tank No.	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Initial Pop.	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
Final Pop.	19	17	20	19	18	19	19	20	20	16	14	15	19	20	18	19	21	19
% Mortality	5	15	0	5	10	5	5	0	0	20	30	25	5	0	10	5	0	5
Day 1																		
2																		
3																		
4																		
5										1								
6											2							
7										1	2	3						
8										2	1	2						
9		1									1							
10																		
11	1															1		
12													1		1			
13					1										1			
14							1											
15																		
16		2																
17																		
18																		
19																		
20																		
21				1	1													
22																		1
23																		
24																		
25																		
26																		
27					1													
28																		
29																		
30																		
31																		
32																		
33																		
34																		
Total Count	1	3	0	1	2	1	1	0	0	4	6	5	1	0	2	1	0	1
Median Day	11	16	-	21	17	27	14	-	-	8	7	7	12	-	12	11	-	22

	Non-Chlorinated: Median Percent Mortality			
Dilution	Primary	Chem. Floc.	Bio. Second.	Control SW
30:1	0	10	5	--
100:1	5	5	5	--
300:1	5	0	0	--
Infinite	--	--	--	1

In this case, there was no suggestion of systematic changes in these low mortality values with either degree of treatment or dilution.

Corresponding values for the chlorinated effluents are listed below:

	Chlorinated: Median Percent Mortality			
Dilution	Primary	Chem. Floc.	Bio. Second.	Control SW
30:1	100	85	25	--
100:1	0	15	5	--
300:1	0	5	5	--
Infinite	--	--	--	1

Discounting the 30:1 dilution values for suspected overchlorination as discussed above, there does appear to be a pattern of decreased mortality with increased treatment and dilution.

As described above, at the end of the experiment 31 surviving specimens were sampled for histopathology. The consultant histopathologist, Dr. Richard Wolke, reported finding abnormal hypertrophy of the atrial macrophase system in all treatments including controls, but most severely in fish which survived in the chlorinated chemical treatment tanks at a 1:30 dilution. Also, one control fish which had minimal atrial macrophase hypertrophy had severe bacterial septicemia. Otherwise, there was no relation between histopathological changes and sewage treatments. In addition, weight and length measurements showed no significant differences between control fish and fish exposed to any treatment.

The results of the bioaccumulation measurements for trace metals and chlorinated hydrocarbons are presented in Tables 62 and 63, respectively. Inspection of the metals data indicates that, relative to the average control values, only for copper is there an indication of elevated whole-body concentrations for the effluent-exposed specimens, and the maximum percentage increase over the control value was only about 50 percent. Further, there did not appear to be any correlation between body burden of a metal and degree of treatment.

In contrast, for PCB 1254 (but not PCB 1016 or total chlorinated pesticides) all of the concentrations measured in the

TABLE 62. CONCENTRATIONS (mg/kg dry wt.) OF TRACE METALS IN WHOLE ORGANISM COMPOSITES OF SPECIMENS COLLECTED AT THE END[a] OF STABEL EXPERIMENTS III (*Cyprinodon variegatus*) AND IV (*Penaeus duorarum*), 30:1 DILUTION

Treatment	Code	Cd	Cr	Cu	Pb	Zn
Experiment III: Sheepshead Minnow (*Cyprinodon variegatus*)						
Pre-Expt. Control	M24	0.69	0.34	5.7	0.08	81
Seawater Control	M31	0.07	0.34	5.2	0.11	77
Primary Settling	M26	0.01	0.18	8.2	ND[b]	75
Chem. Floc.	M30	0.12	0.47	7.2	0.14	80
Chem. Floc.-Chlor.	M29	0.05	0.39	7.6	0.13	78
Bio. Second.	M28	0.07	0.53	7.7	0.08	80
Bio. Second.-Chlor.	M27	NA[c]	NA	8.2	0.22	74
Experiment IV: Pink Shrimp (*Penaeus duorarum*)						
Pre-Expt. Control	M44	0.66	0.58	86	0.03	57
Seawater Control	M53	0.17	0.72	120	0.15	60
Primary Sett.-Chlor.	M48	NA	NA	180	0.19	68
Chem. Floc.	M52	NA	NA	180	0.27	72
Chem. Floc.-Chlor.	M51	0.25	0.37	120	0.04	58
Bio. Second.	M50	0.91	0.29	83	0.06	46
Bio. Second.-Chlor.	M49	0.28	1.1	120	0.09	69

---

[a] Except for Pre-Experiment Control samples.

[b] Not detected.

[c] Not analyzed.

TABLE 63. CONCENTRATIONS (mg/kg dry wt.) OF CHLORINATED HYDROCARBONS IN WHOLE ORGANISM COMPOSITES OF SPECIMENS COLLECTED AT THE END[a] OF STABEL EXPERIMENTS III (*Cyprinodon variegatus*) and IV (*Penaeus duorarum*), 30:1 DILUTION

Treatment	Code	Total Chlor. Pesticides	Polychlorinated Biphenyls 1016	1254	Total
Experiment III: Sheepshead Minnow (*Cyprinodon variegatus*)					
Pre-Expt. Control	P24	(No data were located)			
Seawater Control	P31	0.35	0.21	0.71	0.92
Primary Settling	P26	0.40	0.21	1.5	1.7
Chem. Floc.	P30	0.28	0.35	0.88	1.2
Chem. Floc.-Chlor.	P29	0.36	0.28	1.5	1.8
Bio. Second.	P28	0.41	0.36	1.3	1.7
Bio. Second.-Chlor.	P27	0.81	0.18	1.5	1.7
Experiment IV: Pink Shrimp (*Penaeus duorarum*)					
Pre-Expt. Control	P44	0.13	0.10	0.40	0.50
Seawater Control	P47	0.28	0.25	1.2	1.4
Primary Sett.-Chlor.	P45	1.6	0.28	0.55	0.83
Chem. Floc.	P51	0.29	0.29	0.78	1.1
Chem. Floc.-Chlor.	P50	0.22	0.29	0.85	1.1
Bio. Second.	P49	0.26	0.33	1.1	1.4
Bio. Second.-Chlor.	P48	0.21	0.20	0.74	0.94

---

[a] Except for Pre-Experiment Control samples.

exposed specimen composite samples (0.88 to 1.5 mg/kg dry wt.) exceeded the single control value (0.71) measured in the composite of specimens collected from the seawater control tanks. Again, there was no apparent correlation between PCB whole body concentration and degree of treatment. Further, application of an approximate wet weight:dry weight ratio of 4:1 yields an estimated maximum whole body concentration for total PCB of less than 0.5 mg/kg wet weight, an order-of-magnitude below the FDA guidelines of 5 mg/kg wet weight (ppm).

Experiment IV

Experiment IV was conducted between April 16 and May 22, 1980 using pink shrimp (*Penaeus duorarum*). The mortality results are presented in Tables 64-67. These animals proved to be a very satisfactory bioeffects species except that any animal which died was soon consumed by its cohabitants and therefore not recovered for analysis. Mortality began within two to four days of exposure to the non-chlorinated and chlorinated primary settled wastewater at 30:1 dilution (Table 65) and 75-100 percent of these animals died over the course of the experiments. The causative agent must have been at a critical concentration in the non-chlorinated primary wastewater at this dilution since low mortality was observed at greater dilutions. The median mortality values are summarized below.

	Non-Chlorinated: Median Percent Mortality			
Dilution	Primary	Chem. Floc.	Bio. Second.	Control SW
30:1	100	5	5	--
100:1	5	10	5	--
300:1	5	0	5	--
Infinite	--	--	--	5

	Chlorinated: Median Percent Mortality			
Dilution	Primary	Chem. Floc.	Bio. Second.	Control SW
30:1	80	25	90	--
100:1	10	10	10	--
300:1	5	5	10	--
Infinite	--	--	--	5

With the exception of the primary 30:1 case, there was no indication of a substantial increase in median mortality (over the control value of 5 percent) for any of the three non-chlorinated treatments. For the chlorinated treatments, substantial increases in mortality over the control level were observed for all three treatments at the lowest (30:1) dilution, with mortality generally decreasing as dilution increased. However, most of the drop in mortality occurred between 30:1 to 100:1 dilution.

TABLE 64. STABEL EXPERIMENT IV: OBSERVED MORTALITY VS. TIME FOR Penaeus duorarum EXPOSED TO CONTROL SEAWATER

Tank No.		1	2	3	5	6
Initial Pop.		20	20	20	20	20
Final Pop.		20	19	20	19	18
% Mortality		0	5	0	5	10
Day	1					
	2					
	3					
	4					
	5					
	6					
	7					
	8					
	9					
	10					
	11					
	12					
	13					
	14					
	15					
	16					
	17					
	18					
	19					
	20					
	21					
	22					
	23					
	24					
	25					
	26					
	27					
	28					
	29					
	30					
	31					
	32					
	33					
	34					
	35					
Total Count		0	0	0	0	0
Median Day		-	-	-	-	-

TABLE 65. STABEL EXPERIMENT IV: OBSERVED MORTALITY VS. TIME FOR *Penaeus duorarum* EXPOSED TO WASTEWATER TREATED BY THE PRIMARY SETTLING PROCESS

	Non-Chlorinated									Chlorinated								
Dilution	30:1			100:1			300:1			30:1			100:1			300:1		
Tank No.	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Initial Pop.	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
Final Pop.	0	0	1	19	17	20	19	19	17	2	5	4	18	18	20	18	19	20
% Mortality	100	100	95	5	15	0	5	5	15	90	75	80	10	10	0	10	5	0
Day 1																		
2		1																
3			1															
4	1	2	3	1						7								
5	1									1								
6	6	1								3								
7	10	1	4							6	2	2						
8	3	7	1							1	2	1						
9			1															
10		1	2							1								
11		1	1															
12																		
13			1															
14		1									2	1						
15											1	2						
16																		
17																		
18																		
19																		
20																		
21																		
22																		
23												1						
24			3															
25																		
26			1									1						
27																		
28																		
29																		
30		2										1						
31																		
32																		
33																		
34																		
35																		
Total Count	21	17	18	1	0	0	0	0	0	19	7	8	0	0	0	0	0	0
Median Day	7	8	8	4	-	-	-	-	-	6	8	15	-	-	-	-	-	-

TABLE 66. STABEL EXPERIMENT IV: OBSERVED MORTALITY VS. TIME FOR *Penaeus duorarum* EXPOSED TO WASTEWATER TREATED BY THE CHEMICAL FLOCCULATION PROCESS

	Non-Chlorinated									Chlorinated								
Dilution	30:1			100:1			300:1			30:1			100:1			300:1		
Tank No.	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Initial Pop.	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
Final Pop.	14	19	20	18	20	18	19	20	20	19	15	14	19	18	18	16	19	19
% Mortality	30	5	0	10	0	10	5	0	0	5	25	30	5	10	10	20	5	5
Day 1																		
2																		1
3										1								
4	4																	
5												1		1				
6																		
7																		
8																		
9						1												
10										1								
11																		
12																		
13																		
14																		
15																		
16																		
17																		
18																		
19																		
20																		
21																		
22																		
23																		
24																		
25																		
26																		
27												1			1			
28																2		
29																	1	
30																		
31											1							
32																		
33																		
34																		
35																		
Total Count	4	0	0	0	0	1	0	0	0	2	1	2	0	1	1	2	1	1
Median Day	4	-	-	-	-	9	-	-	-	6	31	16	-	5	27	28	29	2

TABLE 67. STABEL EXPERIMENT IV: OBSERVED MORTALITY VS. TIME FOR *Penaeus duorarum* EXPOSED TO WASTEWATER TREATED BY THE BIOLOGICAL SECONDARY PROCESS

	Non-Chlorinated									Chlorinated								
Dilution	30:1			100:1			300:1			30:1			100:1			300:1		
Tank No.	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Initial Pop.	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
Final Pop.	19	19	18	19	19	19	19	19	19	1	2	3	18	18	18	18	17	20
% Mortality	5	5	10	5	5	5	5	5	5	95	90	85	10	10	10	10	15	0
Day 1																		
2																		
3																		
4																		
5																		
6																		
7																		
8																		
9																		
10																		
11																		
12																		
13																	1	
14																		
15												2						
16												1						
17											2							
18																		
19													1					
20																		
21																		
22										1								
23																		
24																		
25																		
26																		
27										3								
28										9	6	9			1			
29										4	3	3						
30																		
31																		
32																		
33																		
34																		
35																		
Total Count	0	0	0	0	0	0	0	0	0	17	11	15	1	0	1	0	1	0
Median Day	-	-	-	-	-	-	-	-	-	28	28	28	19	-	28	-	13	-

As shown by the whole-body concentrations of trace metals and chlorinated hydrocarbons measured in the composite samples of pink shrimp (Tables 62 and 63), there is no indications that these contaminants were bioaccumulatd substantially over the seawater control values.

A record of epiphytic growth on the inside standpipe of the experiment tanks was also made at the termination of this experiment. Growth was rated using a scale from 0 to 4. Growth consisted mainly of two types of translucent tunicates and some calcareous tubed serpulid polychaetes. Growth was totally inhibited in all dilutions of chlorinated secondary treatments, 30:1 chlorinated chemical, 30:1 and 100:1 chlorinated primary, 30:1 non-chlorinated primary, and 300:1 non-chlorinated chemical treatments. In contrast, prolific growth occurred in 300:1 non-chlorinated primary treatment dilutions. Moderate growth appeared on all other tanks including controls. Thus, it appears that the increased nutrient supply of treated sewage promoted growth of these organisms, but that this situation was counteracted by both chlorinated and the toxicity of the non-chlorinated, least-diluted (30:1) effluents from the primary settling and chemical flocculation processes.

Experiment V

Experiment V was conducted between June 10 and August 3, 1980, using juvenile high hats (Equetus acuminatus) which were spawned and reared at Ocean Farming Systems, Tavernier, Florida. The results are presented in Tables 68-71. However, the exposure to the diluted effluents was short, and no record of the final population was located. Thus, for these tests the median values for total body count have been summarized below for the non-chlorinated and chlorinated cases.

	Non-Chlorinated: Total Body Count (Median)			
Dilution	Primary	Chem. Floc.	Bio. Second.	Control SW
30:1	8	6	1	--
100:1	4	6	6	--
300:1	12	6	7	--
Infinite	--	--	--	4

	Chlorinated: Total Body Count (Median)			
Dilution	Primary	Chem. Floc.	Bio. Second.	Control SW
30:1	20	8	17	--
100:1	6	3	10	--
300:1	2	6	7	--
Infinite	--	--	--	4

These results suggest that, at the lowest dilution (30:1), there was substantially higher mortality with chlorination than

TABLE 68. STABEL EXPERIMENT V: OBSERVED MORTALITY VS. TIME FOR *Equetus acuminatus* EXPOSED TO CONTROL SEAWATER

Tank No.	1	2	3	5
Initial Pop.[a]	20	20	20	20
Day 1				
2				
3				
4				
5				
6				
7				
8				
9				
10[b]				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
25				
26				
27				
28				
29				
30				
31				
32				
33				
34				
35				
36				
37	2			1
38				
39		1		1
40	2	1		
41		1		
42				
43	1	1		
44	2			
45				
46				
47				
48		1		
49				
50				
51				
52				
53	1	1		
54		1		
Total Count	8	7	0	2
Median Day	42	43	-	38

---

[a] No record of Final Population located.

[b] Last day of wastewater exposure.

TABLE 69. STABEL EXPERIMENT V: OBSERVED MORTALITY VS. TIME FOR *Equetus acuminatus* EXPOSED TO WASTEWATER TREATED BY THE PRIMARY SETTLING PROCESS

	Non-Chlorinated									Chlorinated								
Dilution	30:1			100:1			300:1			30:1			100:1			300:1		
Tank No.	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Initial Pop.[a]	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
Day 1	1									20	19		2	16				
2											1	3	1	1				
3																		
4																		
5																		
6			2															
7			8															
8		1	5									1						
9												3						
10[b]																		
11				1														
12																		
13																		
14																		
15																		
16																		
17																		
18																		
19																		
20																		
21																		
22																		
23																		
24					1													
25																		
26			1					6							1			
27								6								1		
28								4										
29								1[c]										
30																		
31																		
32																		
33																		
34																		
35																		
36							1											
37													1					
38					1		1						1					
39		2		1														
40	1	1		1			3											
41		1			1								1			3		
42				1			3											
43																		
44							1									1		
45																	1	
46		1		1														
47				1			1											
48		2			1													
49				1			1											
50				2			1											
51																		
52																		
53				1												1		
54																	1	
Total Count	2	8	16	10	4	0	12	17	0	20	20	7	6	17	1	6	2	0
Median Day	20	40	7	46	40	-	42	27	-	1	1	8	20	1	26	41	50	-

---

[a] No record of Final Population located.
[b] Last day of wastewater exposure.
[c] All speciments in Tank 2 dead by Day 29.

TABLE 70. STABEL EXPERIMENT V: OBSERVED MORTALITY VS. TIME FOR *Equetus acuminatus* EXPOSED TO WASTEWATER TREATED BY THE CHEMICAL FLOCCULATION PROCESS

	Non-Chlorinated									Chlorinated								
Dilution	30:1			100:1			300:1			30:1			100:1			300:1		
Tank No.	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Initial Pop.[a]	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
Day 1										4	1							
2										1	3							
3																		
4																		
5						1			1									
6																		
7			1															
8												1						
9			1								16	5			1			
10[b]												2						
11																		
12																		
13																		
14																		
15																		
16																		
17																		
18																		
19																		
20																		
21																		
22																		
23																		
24																		
25																		
26																		
27																		
28																		
29																		
30				1														
31																		
32																		
33																		
34																		
35									1									
36							1											
37		1					1											
38	1	4		1	2		2	2					2				2	
39	1				2											1		
40		3		4	2			1					1			1		
41	3	1			3		1	1					2	1		1	1	
42					1													
43					1					1								
44																		
45								2		1			1					
46								1						1		1		
47							1									1		
48								1								1		
49					1													
50		1						1								1		
51																		
52																		
53														1				
54													3			1		
Total Count	6	10	2	6	12	1	6	9	2	7	20	8	9	3	1	8	3	-
Median Day	41	39	8	40	40	5	38	45	20	1	9	9	41	46	9	46	38	-

---

[a] No record of Final Population located.

[b] Last day of wastewater exposure.

TABLE 71. STABEL EXPERIMENT V: OBSERVED MORTALITY VS. TIME FOR *Equetus acuminatus* EXPOSED TO WASTEWATER TREATED BY THE BIOLOGICAL SECONDARY PROCESS

	Non-Chlorinated									Chlorinated								
Dilution	30:1			100:1			300:1			30:1			100:1			300:1		
Tank No.	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Initial Pop.[a]	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
Day 1																		
2												7						
3												2						
4																		
5																		
6						1				20	17	3						
7												1						
8												1						
9												1						
10																		
11																		
12																		
13																		
14																		
15																		
16																		
17																		
18																		
19																		
20																		
21																		
22																		
23																		
24																		
25																		
26																		
27																		
28	1																1	
29																		
30													1					
31																		
32																		
33																		
34																		
35																		
36														2				
37	1							1									5	
38	1			1			1	1					1	1			1	
39				1									1					
40	1	1		1	1		4	1					2	5		1	2	
41				1	2								3				1	
42	1						1											
43																2	1	
44			1											2		2		
45				2	1								1			1		
46																		
47	1							1										
48					1													
49								1								1	1	
50				2	1													
51																		
52																		
53							1							1				
54							1	2					1	2				
Total Count	6	1	1	8	6	1	8	7	0	20	17	15	10	13	0	7	12	0
Median Day	39	40	44	43	43	6	40	47	-	6	6	3	40	40	-	44	38	-

---

[a] No record of Final Population located.

without. Further, in the case of chlorination, mortality generally decreased with increasing dilution.

It is instructive also to examine the median time to death values, as summarized below.

	Non-Chlorinated: Median Days to Death			
Dilution	Primary	Chem. Floc.	Bio. Second.	Control SW
30:1	20	39	40	--
100:1	43	40	43	--
300:1	34	38	44	--
Infinite	--	--	--	42

	Chlorinated: Median Days to Death			
Dilution	Primary	Chem. Floc.	Bio. Second.	Control SW
30:1	1	9	6	--
100:1	20	41	40	--
300:1	46	42	41	--
Infinite	--	--	--	42

These results indicate that, for effluents of all three treatments at the lowest dilution (30:1), and for the intermediate dilution (100:1) of primary effluent, median times to death were substantially shorter with clorination than without. Increasing the dilution from 30:1 to 100:1 greatly increased the survival time of the specimens exposed to the chlorinated effluent from all three treatments. The greatest benefit was seen in the chlorinated primary case, where going from 30:1 to 100:1 dilution increased the median time to death 20-fold (from 1 to 20 days).

However, increasing the dilution by another factor of 3 (from 100:1 to 300:1) increased the survival period about 2-fold, an order of magnitude less improvement. In the case of the chlorinated chemical flocculation and biological secondary effluents, no further increase in median survival time was observed in increasing the dilution from 100:1 to 300:1

SECTION 10

STABEL EXPERIMENTS WITH CORALS

INTRODUCTION

The coast between Miami and Palm Beach is characterized by sand beaches of immense economic importance to the local tourist industry and by a series of submerged offshore reefs. The reefs are colonized by a hard bottom fauna of which gorgonians (soft corals), scleractinian corals and sponges are the dominant seasile macrobenthos. In this study, corals were selected as the primary test organisms of the reef community because they play a dominant role in the ecology of the complex reef ecosystem, in nutrient cycling and in reef growth. Corals and gorgonians are key elements of benthic primary production because of their symbiosis with unicellular algae (Goreau, 1959; Pearse and Muscatine, 1971; Taylor, 1973). Any effects of effluents on the coral community are likely to be reflected throughout the reef ecosystem. This section of the report presents the initial results of effluent effects on two species of reef corals, *Montastrea cavernosa* and *Dichocoenia stokesii*.

PROCEDURES

Corals were collected by divers from offshore reefs located approximately 3 km from the STABEL facility. Individual corals were carefully dislodged from the reef with a pry-bar and transported to STABEL in 35-liter styrofoam containers. Specimens were selected on the basis of size (20-30 cm diameter for head corals) and apparent health from reef areas which appeared unaffected by the Virginia Key sewage outfall. The corals were maintained for up to two weeks in one of the STABEL holding tanks with a continuous supply of Bear Cut seawater. After the two-week acclimation period, only those corals which appeared healthy were used for the exposure experiments. Each specimen was photographed in color before and after each experiment. Four corals were placed in each of the 20 experimental tanks (18 effluent-types and 2 controls) on a plastic mesh rack suspended at about a 40-cm depth. The seawater was gradually enriched with effluent until the predetermined concentration for each tank was reached. The exposure experiments were conducted between March 4 and April 11, 1980 (approximately the same period as for Experiment III).

Corals were monitored daily by visual inspection. Each coral was examined for signs of mortality or stress (abnormal polyp retraction or extension, bleaching due to loss of zoozanthaellae, copious mucus secretion, and infestation by pathogens or "black-line" disease). The 20 test aquaria were photographed at the end of the experiment to document the effect of fouling by microorganisms and macroalgae.

At the termination of the experiment, the corals were rinsed in filtered seawater, photographed, cleaned of adhering algae, wrapped in aluminum foil and frozen. After partial thawing, each specimen was rinsed with distilled water to remove surface contaminants. Tissue was then removed from a portion of the coral skeleton using a "Water Pik" device which removes coral tissue from the skeleton with a jet of pressurized water. Approximately 100 to 250 ml of distilled water and coral tissue was collected in a plastic bag, strained through 300 um nylon plankton net to remove larger tissue fragments, skeletal debris, etc. and the remaining tissue/water mixture stored in a precleaned glass container. All samples were then stored at 4°C until carbon and trace constituent measurements were made.

Total organic carbon (TOC) was determined for each tissue/water sample to allow quantification of analytical data. TOC content of each sample was determined with a Beckman Model 915 Total Organic Carbon Analyzer which converts all carbon in the sample to CO_2. A standard curve was prepared using stock solutions and a least squares linear regression was performed on all standard determinations. Samples were diluted with distilled water to give values (peak heights) in the range of the standard curve. Distilled water was used as a blank. TOC of each sample was determined 2-5 times and the average peak height was used in the linear regression equation to give TOC. These TOC concentrations then were used to normalize the trace metal, chlorinated hydrocarbon, and micronutrient concentrations measured in the tissue/water mixtures.

The target trace metals were analyzed using a Perkin-Elmer model 403 graphite furnace atomic absorption spectrometer. Chlorinated hydrocarbons were analyzed using a Tracor model 222 gas chromatograph equipped with a Ni-63 electron capture detector. Micronutrients were analyzed on a Technician CSM6 auto analyzer and a Beckman DU Spectrophotometer following methods outlined in Standard Methods (1975). Additional details of analytical procedures used are presented in Appendix D.

RESULTS AND DISCUSSION

Table 72 and Figures 14-37 reveal the relative effects of effluent treatment, chlorination and dilution. These relationships indicate that the level of effluent treatment is the most important factor influencing fouling of the aquaria and potential overgrowth of the coral by algae. Primary treatment resulted in accelerated fouling, regardless of chlorination or dilution.

TABLE 72. RELATIVE FOULING OF EXPOSURE AQUARIA FOR CORAL EXPERIMENTS

	Treatment Process
PS	Primary Settling
CF	Chemical Flocculation
AS	Activated Sludge Bio. Second.
NC	Non-Chlorinated
Cl	Chlorinated

30
100 Dilution Factor
300

Degree of Fouling	
(+)	More than controls
(=)	Similar to controls
(-)	Less than controls

	Treatment					
	Primary		Chem. Floc.		Bio. Second.	
Dilution	Non-Chlor.	Chlor.	Non-Chlor.	Chlor.	Non-Chlor.	Chlor.
30:1	+	+	+	-	+	-
100:1	+	+	+	-	+	-
300:1	+	+	=	-	=	-

Figures 14-19. Primary Settling (PS). Experimental aquaria contained the reef corals *Montastrea cavernosa* and *Dichocoenia stokesii*. All PS aquaria, whether non-chlorinated (NC) or chlorinated (Cl) and regardless of dilution, exhibited more fouling than did either chemical flocculant (CF) or activated sludge (AS) aquaria. Effluent/seawater mixtures at 30:1 dilution were turbid (Figures 14 and 15); note increased water clarity at higher dilutions (Figures 16-19).

Figure 14: PS NC 30

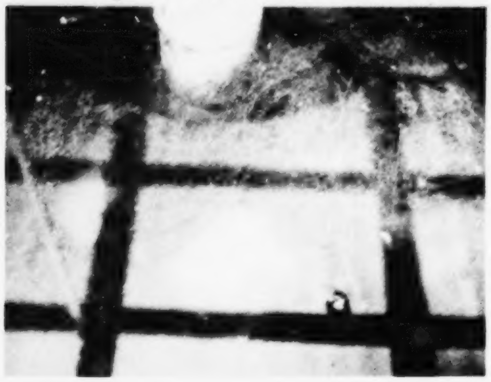


Figure 15: PS C1 30

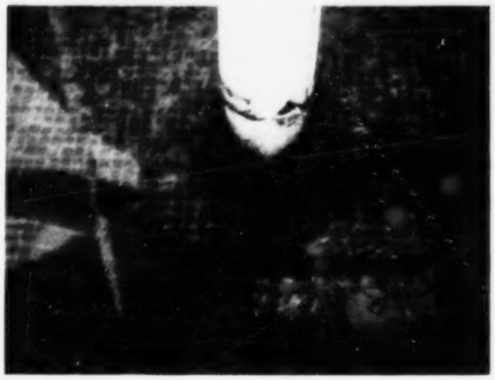


Figure 16: PS NC 100

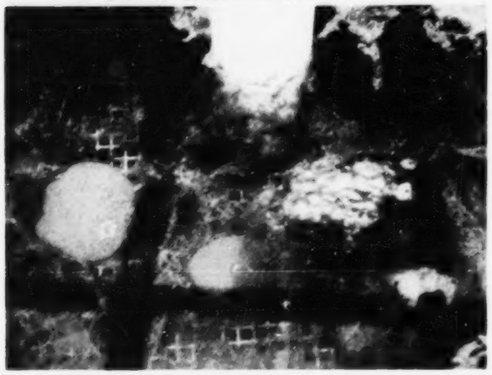


Figure 17: PS C1 100

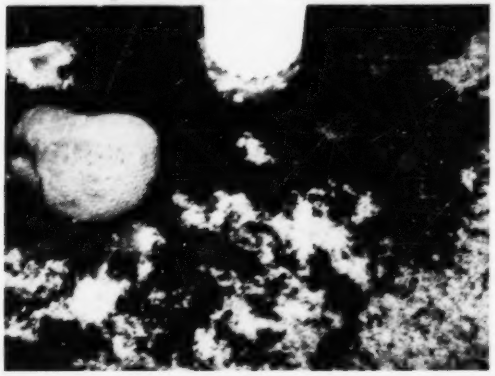


Figure 18: PS NC 300

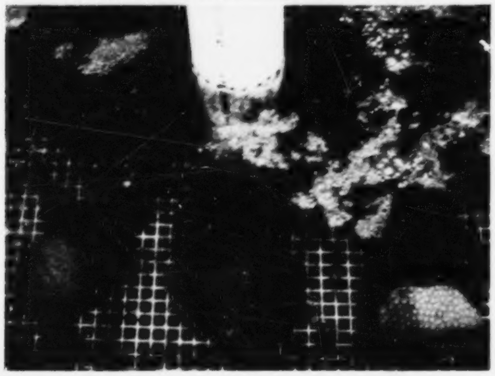
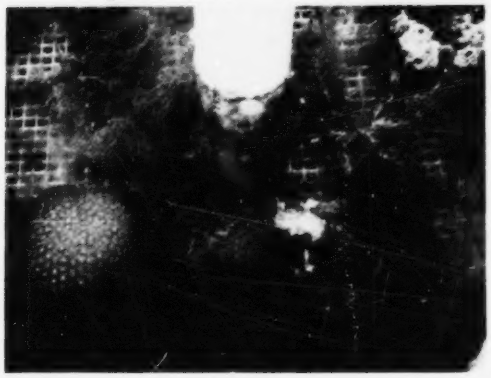


Figure 19: PS C1 300



Figures 20-25. Chemical Flocculation (CF). Chlorination reduced fouling, even at 300:1 dilution (Figure 25). Chlorinated aquaria (Figures 21, 23, 25) are free of macroalgae and surface "scum" layer.

Figure 20: CF NC 30

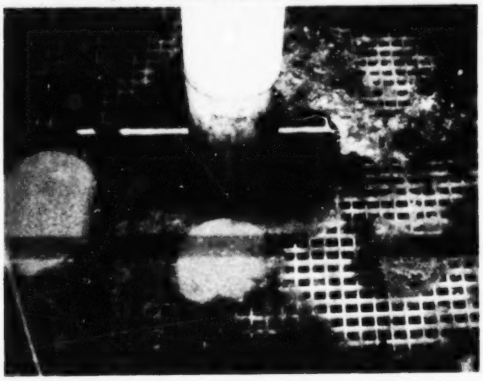


Figure 21: CF Cl 30

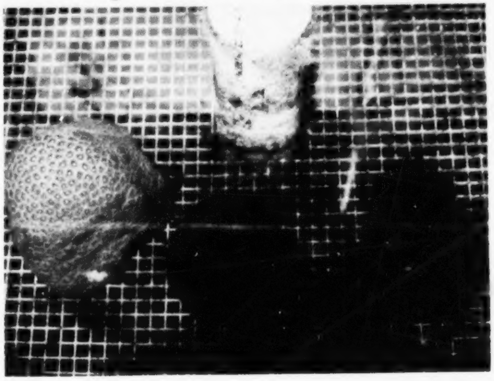


Figure 22: CF NC 100

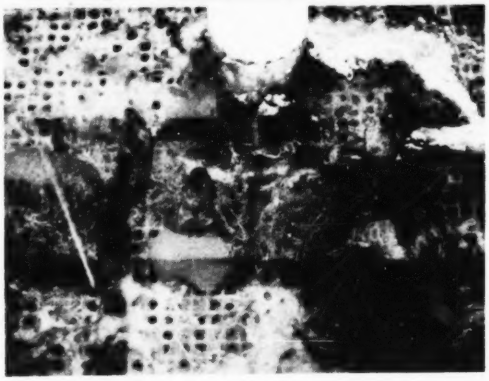


Figure 23: CF Cl 100

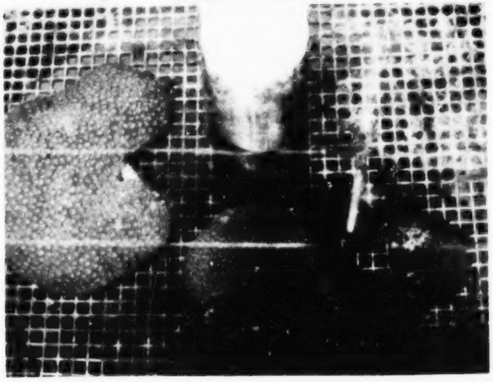


Figure 24: CF NC 300

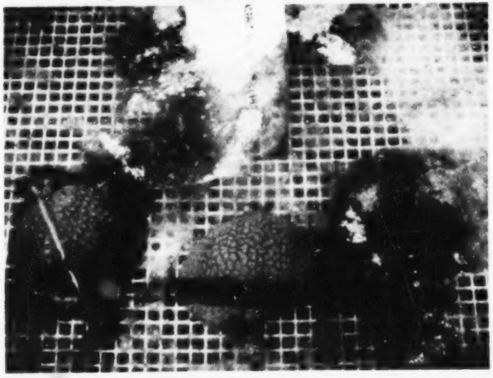
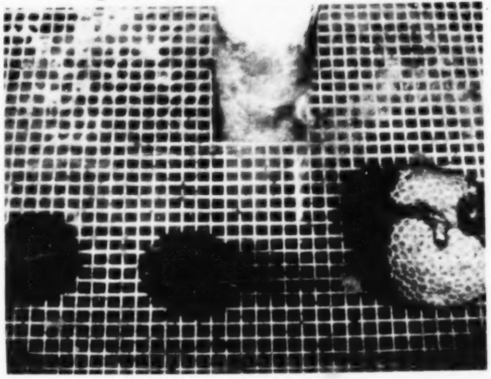


Figure 25: CF Cl 300



Figures 26-31. Activated Sludge Biological Secondary (AS). All chlorinated effluents (Figures 27, 29, 31) fouled less than non-chlorinated effluents (Figures 26, 28, 30).

Figure 26: AS NC 30

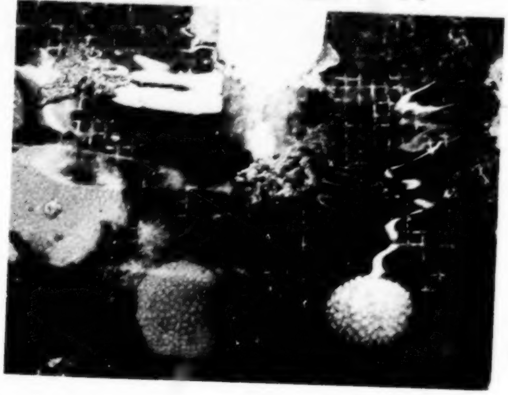


Figure 27: AS Cl 30

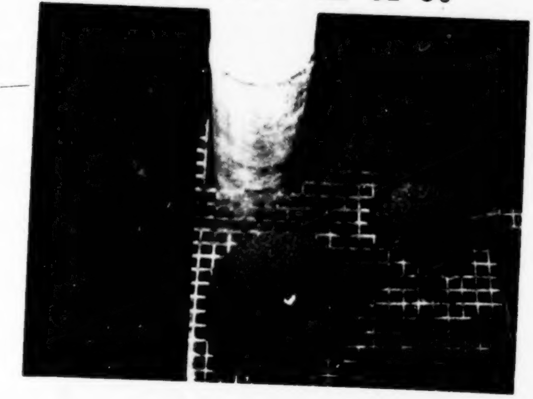


Figure 28: AS NC 100

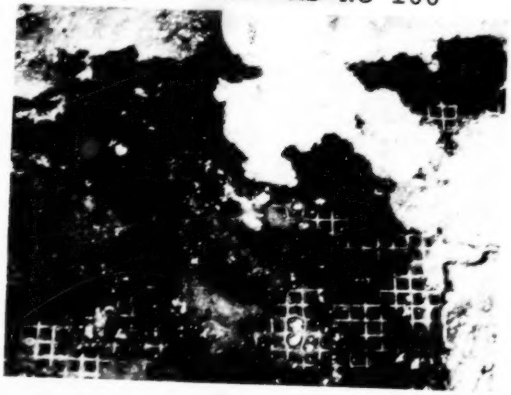


Figure 29: AS Cl 100

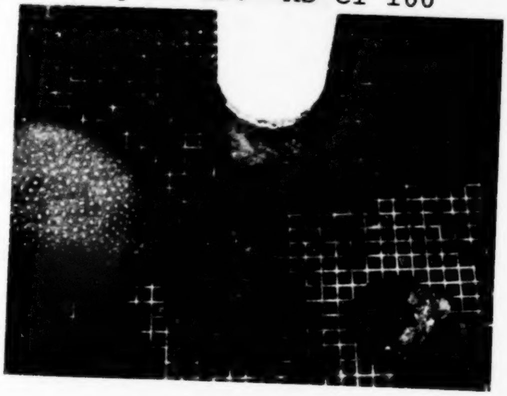


Figure 30: AS NC 300

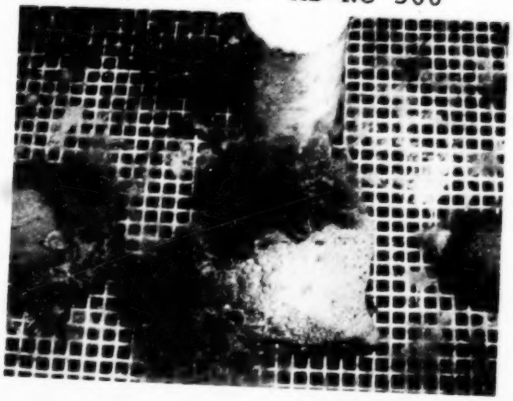
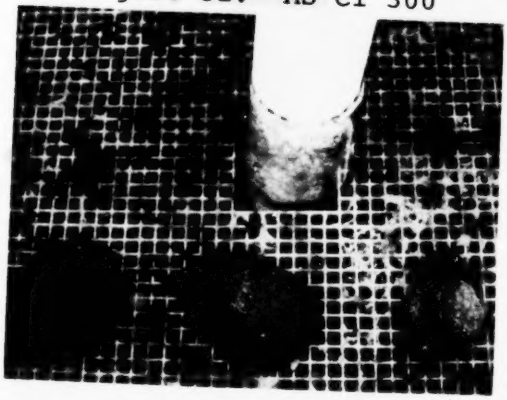


Figure 31: AS Cl 300



Figures 32 and 33. Controls. Filamentous and other algae grew in the control aquaria at a higher rate than in the 30:1 chlorinated effluents treated by chemical flocculant (Figure 21) and activated sludge (Figure 27).

Figure 34. D. stokesii before experiment. Note algae at base of coral. Control.

Figure 35. Same specimen as Figure 34 at the end of experiment; note increase in size of algae. Some control corals also exhibited minor bleaching.

Figure 36. M. cavernosa before experiment. Primary treatment, non-chlorination, 30:1 dilution.

Figure 37. Same coral as in Figure 36 at end of experiment. The coral was completely overgrown by a canopy of filamentous algae and other fouling organisms, later removed to expose the dead white skeleton. From aquarium shown in Figure 14 (coral not visible). This coral was the only fatality of the experiments.

Figure 32: CONTROL

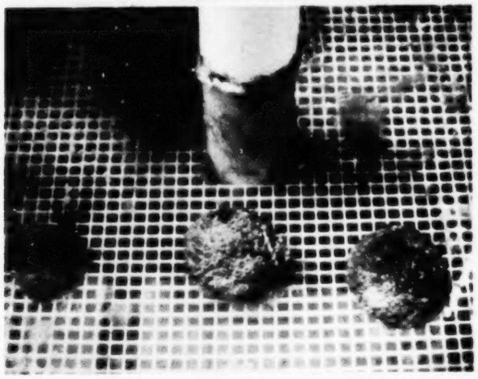


Figure 33: CONTROL

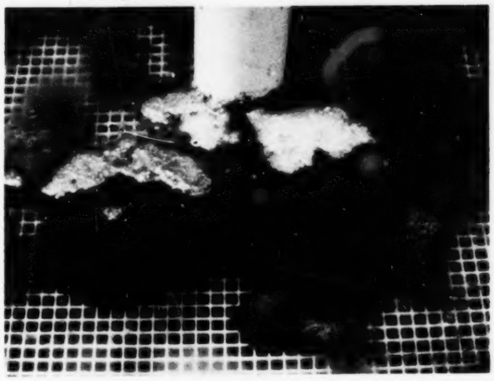


Figure 34

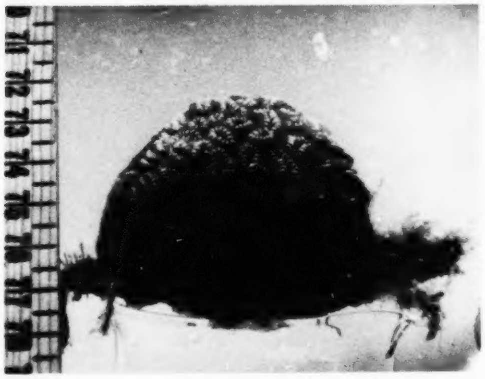


Figure 35

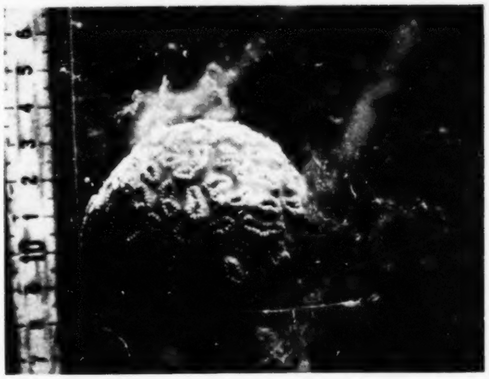


Figure 36

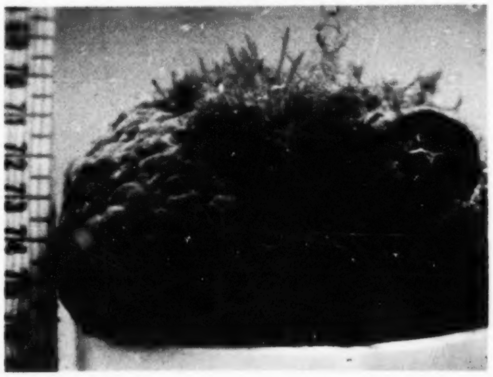
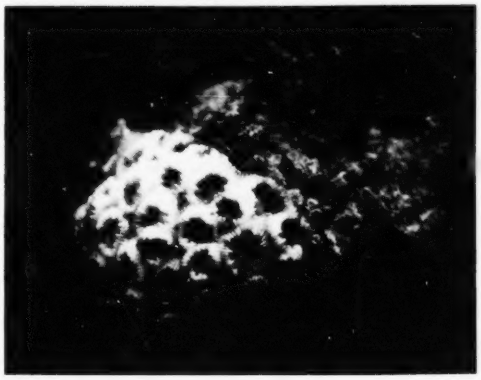


Figure 37



Chlorination is important in the control of fouling, especially in effluents treated which chemical flocculation or activated sludge; regardless of dilution, all chlorinated chemical flocculation (CF) or activated sludge biological secondary (AS) treated effluents resulted in reduced fouling. Compare Figures 20, 22, and 24 (non-chlorinated) to Figures 21, 23, and 25 (chlorinated), and Figures 26, 28, and 30 (non-chlorinated) to Figures 27, 29, and 31 (chlorinated).

Dilution was important in determining the level of fouling in non-chlorinated effluents, especially those treated by chemical flocculation (CF) and activated sludge biological secondary (AS) methods. The 300:1 dilution tanks had considerably less fouling than the 30:1 tanks (compare Figures 24 to 20 and Figures 30 to 26).

The observations on fouling during this experiment should be applied with caution to ocean outfalls, where under field conditions the luxurient growth or fouling algae may be prevented by the grazing activites of reef fishes and invertebrates. Along the southeast Florida coast, as in many other reef environments, fleshy algae may be abundant in adjacent biotopes (seagrass beds, sediment banks, rocky shoreline, etc.), but they are inconspicuous or absent on the reef per se. Close examination of the reef usually reveals that fleshy algae are present, but typically as small plants in crevices, holes, attached to the undersides of corals and in other sheltered locations. During collection of corals for this study, it was noted that most specimens had small unidentifiable algae attached to their bases and other dead portions of the skeleton. After a one to two-week acclimation period in the holding tanks (and in the absence of grazing organisms), they had grown into larger recognizable specimens of Dictyota, Stypopodium, Padina and other algae (see Figures 34-37).

Whether or not ocean outfalls result in eutrophication of the surrounding area to the extent that corals and other sessible benthos are overgrown by algae depends on several factors. Among the most important are the level of nutrient enrichment, the grazing potential of herbivores, light, and the availability of other (not-effluent mediated) nutrients. In Kanehoe Bay, Hawaii, sewage effluents resulted in the replacement of the coral reef community by a luxurient growth of Dictyosphera, a fleshy green macroalgae also common in south Florida (Maragos, 1972; Banner, 1974). Algae are believed to have colonized the coral skeletons after the corals had died from exposure to toxicants, siltation, turbidity or other causes.

This study indicates that coral death may occur as a direct result of overgrowth by algae, thus nutrient rich "non-toxic" effluents may favor the growth of some organisms (e.g., algae) and be indirectly lethal to others (e.g., corals). In the experimental aquaria, overgrowth of corals by algae was in the form of a canopy which gradually covered the corals in heavily fouled aquaria. The algae were attached to the coral bases and to other portions of the skeleton not occupied by polyps, but the canopy was separated from the living corals by up to several centimeters of seawater. The algae canopy, although not in contact with the living coral, decreases seawater circulation at the coral surface, reduces ambient light (required by zooxanthaellae which affect calcification rate and may supply the coral with nutrient metabolites), and prevents zooplankton and other food organisms from reaching the coral polyps. The extreme difference in growth rates (a few cm/yr for head corals vs. as much as 10 cm/wk for some algae) give the algae an overwhelming growth advantage.

The coral soft tissue concentrations of trace metals, chlorinated hydrocarbons, and micronutrients (normalized to TOC content) are listed in Tables 73-82. These data do not indicate any clear trend in concentration for any of these constituents with degree of treatment or dilution.

TABLE 73. CADMIUM (ug/g TOC) IN SOFT TISSUES OF CORALS *D. stokesii* AND *M. cavernosa* EXPOSED TO DILUTED STABEL WASTEWATERS

	Non-Chlorinated			Chlorinated		
Species Code	30:1	100:1	300:1	30:1	100:1	300:1
	PRIMARY					
D	ND	ND	4.1	ND	ND	3.9
D	ND	ND	3.6	ND	ND	6.8
M	1.3	ND	5.8	2.3	ND	9.6
	CHEMICAL FLOC.					
D	ND	8.6	2.8	1.1	ND	ND
D	ND	ND	1.6	ND	ND	0.1
M	1.5	ND	ND	ND	11	4.5
	BIO. SECONDARY					
D	ND	0.3	ND	3.5	ND	30
D	ND	ND	ND	ND	ND	ND
M	ND	1.3	ND	ND	ND	4.4
	SEAWATER CONTROL					
D	2.0	2.5				
D	ND	ND				
M	ND	ND				

TABLE 74. CHROMIUM (ug/g TOC) IN SOFT TISSUES OF CORALS D. stokesii AND M. cavernosa EXPOSED TO DILUTED STABEL WASTEWATERS

Species Code	Non-Chlorinated 30:1	Non-Chlorinated 100:1	Non-Chlorinated 300:1	Chlorinated 30:1	Chlorinated 100:1	Chlorinated 300:1
	PRIMARY					
D	5.0	7.1	33	24	16	17
D	ND	ND	26	ND	ND	9.1
M	19	24	16	54	18	98
	CHEMICAL FLOC.					
D	ND	8.2	6.6	11	3.0	17
D	ND	5.1	ND	1.8	0.8	9.5
M	0.9	12	16	ND	40	55
	BIO. SECONDARY					
D	7.7	44	5.1	17	5.1	2.5
D	7.1	4.3	25	ND	4.2	ND
M	23	22	16	32	16	25
	SEAWATER CONTROL					
D	16	7.0				
D	2.5	2.3				
M	80	23				

TABLE 75. COPPER (ug/g TOC) IN SOFT TISSUES OF CORALS *D. stokesii* AND *M. cavernosa* EXPOSED TO DILUTED STABEL WASTEWATERS

	Non-Chlorinated			Chlorinated		
Species Code	30:1	100:1	300:1	30:1	100:1	300:1
	PRIMARY					
D	59	53	110	ND	140	100
D	56	37	150	64	ND	100
M	110	120	160	230	240	230
	CHEMICAL FLOC.					
D	ND	70	60	120	62	110
D	38	54	99	79	86	88
M	110	130	100	70	210	300
	BIO. SECONDARY					
D	100	87	53	100	77	38
D	42	79	170	57	170	42
M	180	180	130	170	160	150
	SEAWATER CONTROL					
D	63	100				
D	60	18				
M	33	230				

TABLE 76. LEAD (ug/g TOC) IN SOFT TISSUES OF CORALS *D. stokesii* AND *M. cavernosa* EXPOSED TO DILUTED STABEL WASTEWATERS

	Non-Chlorinated			Chlorinated		
	30:1	100:1	300:1	30:1	100:1	300:1
Species Code	PRIMARY					
D	ND	ND	ND	ND	ND	ND
D	ND	ND	ND	ND	ND	7.3
M	ND	ND	ND	ND	2.6	11
	CHEMICAL FLOC.					
D	ND	ND	ND	ND	ND	ND
D	ND	ND	ND	ND	ND	ND
M	ND	ND	1.4	ND	ND	ND
	BIO. SECONDARY					
D	ND	ND	ND	ND	ND	ND
D	ND	ND	ND	ND	ND	ND
M	ND	ND	ND	ND	ND	ND
	SEAWATER CONTROL					
D	ND	ND				
D	ND	ND				
M	ND	ND				

TABLE 77. ZINC (ug/g TOC) IN SOFT TISSUES OF CORALS D. stokesii AND M. cavernosa EXPOSED TO DILUTED STABEL WASTEWATERS

Species Code	Non-Chlorinated 30:1	Non-Chlorinated 100:1	Non-Chlorinated 300:1	Chlorinated 30:1	Chlorinated 100:1	Chlorinated 300:1
	PRIMARY					
D	290	320	460	420	200	440
D	310	250	360	280	290	400
M	510	390	620	990	590	420
	CHEMICAL FLOC.					
D	300	310	360	300	180	360
D	190	410	230	400	200	330
M	280	8	670	460	1040	830
	BIO. SECONDARY					
D	340	300	120	290	290	210
D	220	210	360	130	770	170
M	940	670	660	250	990	580
	SEAWATER CONTROL					
D	270	250				
D	390	110				
M	290	450				

TABLE 78. NO_2-N (ug/g TOC) IN SOFT TISSUES OF CORALS *D. stokesii* AND *M. cavernosa* EXPOSED TO DILUTED STABEL WASTEWATERS

	Non-Chlorinated			Chlorinated		
	30:1	100:1	300:1	30:1	100:1	300:1
Species Code	PRIMARY					
D	ND	43	140	ND	ND	61
D	47	44	38	ND	27	56
M	45	ND	ND	ND	110	106
	CHEMICAL FLOC.					
D	ND	43	34	58	64	46
D	ND	ND	50	59	91	59
M	ND	ND	63	ND	93	86
	BIO. SECONDARY					
D	33	71	39	92	65	45
D	51	62	90	41	50	34
M	82	75	140	80	ND	ND
	SEAWATER CONTROL					
D	40	107				
D	48	ND				
M	ND	ND				

TABLE 79. NO_3-N (ug/g TOC) IN SOFT TISSUES OF CORALS *D. stokesii* AND *M. cavernosa* EXPOSED TO DILUTED STABEL WASTEWATERS

	Non-Chlorinated			Chlorinated		
	30:1	100:1	300:1	30:1	100:1	300:1
Species Code	PRIMARY					
D	190	390	3700	210	280	710
D	640	390	430	180	22	560
M	400	310	380	590	890	850
	CHEMICAL FLOC.					
D	140	440	390	570	720	470
D	220	200	450	770	1200	620
M	240	260	550	380	840	750
	BIO. SECONDARY					
D	350	730	410	730	980	490
D	930	500	1090	700	740	570
M	720	860	120	780	430	910
	SEAWATER CONTROL					
D	460	530				
D	570	260				
M	350	710				

TABLE 80. PO_4-P (ug/g TOC) IN SOFT TISSUES OF CORALS _D. stokesii_ AND _M. cavernosa_ EXPOSED TO DILUTED STABEL WASTEWATERS

Species Code	Non-Chlorinated 30:1	Non-Chlorinated 100:1	Non-Chlorinated 300:1	Chlorinated 30:1	Chlorinated 100:1	Chlorinated 300:1
	PRIMARY					
D	2.5	2.8	1.2	3.9	3.3	1.4
D	2.6	1.0	2.2	4.4	1.9	2.0
M	4.7	2.1	3.1	1.8	2.5	3.1
	CHEMICAL FLOC.					
D	1.2	1.5	2.2	2.5	1.9	0.9
D	3.2	3.5	1.3	4.2	1.0	1.0
M	5.0	1.8	1.5	3.4	2.5	1.3
	BIO. SECONDARY					
D	2.6	2.4	1.0	1.3	2.5	1.0
D	1.9	1.8	2.8	1.8	1.8	1.8
M	2.4	4.3	2.1	1.2	1.1	1.6
	SEAWATER CONTROL					
D	1.8	1.1				
D	0.8	1.1				
M	1.9	2.4				

TABLE 81. TOTAL CHLORINATED PESTICIDES (ug/g TOC) IN SOFT TISSUES OF CORALS *D. stokesii* AND *M. cavernosa* EXPOSED TO DILUTED STABEL WASTEWATERS

	Non-Chlorinated			Chlorinated		
	30:1	100:1	300:1	30:1	100:1	300:1
Species Code	PRIMARY					
M		1.9		2.8		4.8
D	2.0	0.3		1.4		0.5
	CHEMICAL FLOC.					
M				8.3		
D	0.8					
	BIO. SECONDARY					
M	4.6				2.2	
D	0.5				0.4	

TABLE 82. TOTAL PCB (ug/g TOC) IN SOFT TISSUES OF CORALS _D. stokesii_ AND _M. cavernosa_ EXPOSED TO DILUTED STABEL WASTEWATERS

Species Code	Non-Chlorinated 30:1	Non-Chlorinated 100:1	Non-Chlorinated 300:1	Chlorinated 30:1	Chlorinated 100:1	Chlorinated 300:1
	PRIMARY					
M		6.4		21		36
D	23	22		4.1		12
	CHEMICAL FLOC.					
M				9.4		
D	5.5					
	BIO. SECONDARY					
M	44			30		
D	14			6.6		

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APPENDIX A

ENGINEERING DRAWINGS FOR STABEL

The University of Miami Rosenstiel School of Marine and Atmospheric Science Sewage Treatment and Bioeffects Laboratory (STABEL) was designed under EPA Project No. R 804749 by H.J. Ross Associates, Inc., Consulting Engineers of Miami, Florida. A complete set of drawings is on file at the University of Miami Facilities Planning and Construction Office, P.O. Box 248106, Coral Gables, Florida 33124. Photographs of these drawings, and the corresponding set of negatives from which velam originals for blueprint reproduction can be obtained, have been submitted for archiving to the EPA Project Officer, Dr. Donald Baumgartner, Director, Marine Division, U.S. Environmental Protection Agency, Marine Science Center, Newport, Oregon 97365.

The titles of the individual drawings included in this set are listed below:

1. Title Sheet
2. Architectural Floor Plan and Details
3. Architectural Elevations and Details
4. Architectural Sections and Details
5. Architectural Foundation Plan and Details
6. Paving and Grading Site Plan
7. Lighting Plan - Electrical
8. Power Plan - Electrical
9. Process Flow Diagram
10. Equipment Location Plan
11. Process Piping Plan
12. Sections and Details
13. Sections and Details
14. Drainage Plan and Details
15. Equipment and Piping Details
16. Site Work Plan
17. Site Work Details
18. Salt Water System - Plan and Sections
19. Salt Water System - Details
20. Force Main and Grit Chamber - Plan and Section
21. Grit Chamber - Electrical
22. Salt Water System Plans and Details - Electrical
23. Salt Water System Control Diagrams - Electrical.

APPENDIX B

PARTIALLY ANNOTATED BIBLIOGRAPHY

The titles obtained from the computerized literature search as part of the SEFOOS project have been separated into the following categories:

A. Bioassay Procedures
B. Chlorine and Chloramines
C. Detergents
D. Heavy Metals
E. Ntrogenous Compounds
F. Oil and Petrochemicals
G. Pathology and Histology
H. Pesticides, PCB's, and Other Organic Toxicants
I. Sewage and Wastewater
J. Sulfur Compounds
K. Suspended Solids
L. Toxicants - Miscellaneous and General.

These groups of titles are preceded by an alphabetized list of all authors' and co-authors' names, indicating the order within a specific group in which the corresponding citation is presented.

Ababei, L. H34
Abdelghani, A.A. H5
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Alexander, G. V. D60
Allen, G.H. I1
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D15 Birge, W.J., A.G. Westerman, and J.A. Black. 1976. Sensitivity of vertebrate embryos to heavy metals as a criterion of water quality. Phase III. Use of fish and amphibian eggs and embryos as bioindicator organisms for evaluating water quality. Office of Water Research and Technology. Wash., D.C. OWRT-B-044-KY.

D16 Bovee, E.C. 1975. Effects of certain chemical pollutants on small aquatic animals. Office of Water Research and Technology, Wash. D.C. OWRT-A-058-KAN.

D17 Boyden, C.R. 1975. Distribution of some trace metals in Poole Harvor, Dorset. Mar. Pollut. Bull. 6:180-187.

D18 Brown, V.M., T.L. Shaw, and D.G. Shurben. 1974. Aspects of water quality and the toxicity of copper to rainbow trout. Water Res. 8:797-803.

The toxicity of copper to trout depends on the particular natural water used for bioassay. Sewage effluent, organic matter, an amino acid and humic substances were added to water containing CuSO$_4$. The copper complexes thus formed resulted in reduced toxicity.

D19 Brungs, W.A., J.R. Geckler, and M. Gast. 1976. Acute and chronic toxicity of copper to the fathead minnow in a surface water of variable quality. Water Res. 10:37-43.

Static 96 hour bioassays of copper in natural stream water containing sewage determined that if total Cu was measured, LD$_{50}$'s ranged from 1.6 to 21 mg/l, but if free Cu was measured the LD$_{50}$'s were 0.60 to 0.98 mg/l. Thus several factors such as increased hardness, pH and alkalinity resulted in the formation of complexed Cu compounds rather than toxic dissolved Cu.

D20 Buhler, D.R., R.R. Claeys, and B.R. Mate. 1975. Heavy metal and chlorinated hydrocarbon residues in California sea lions (*Zalophus californianus californianus*). J. Fish Res. Board Can. 32:2391-2397.

D21 Calabrese, A., R.A. Greig, F.P. Thurberg. R.A. Robohm, E. Gould and M.W. Newman. 1974. Physiological response of the cunner, *Tautogolabrus adspersus*, to cadmium. NOAA Technical Report NMFS SSRF-681.

D22 Cardwell, R.D., D.G. Foreman, T.R. Payne and D.J. Wilbur. 1976. Acute toxicity of selenium dioxine to freshwater fishes. Arch. Environ. Contam. Toxicol. 4:129-144.

D23 Carmody, D.J., J.B. Pearce, and W.E. Yasso. 1973. Trace metals in sediments of New York Bight. Mar. Pollut. Bull. 4:132-135.

"Concentrations of chromium, copper, lead, nickel and zinc in superficial sediments in New York Bight are ten to a hundred times greater near waste disposal areas than in uncontaminated sediments. Some wastes are transported by currents north-east towards Long Island and south-east along the Hudson Submarine Valley."

D24 Casterline, J.L. and G. Yip. 1975. The distribution and binding of cadmium in oyster, soybean, and rat liver and kidney. Arch. Environ. Contam. Toxicol. 3:319-329.

D25 Chapman, G.A. 1978. Effects of continuous zinc exposure on sockeye salmon during adult-to-smolt freshwater residency. Trans. Am Fish. Soc. 107:828-836.

D26 Chapman, G.A. 1978. Toxicities of cadmium, copper, and zinc to four juvenile stages of chinook salmon and steelhead. Trans. Am. Fish. Soc. 107:841-847.

D27 Chapman, G.A. and D.G. STevens. 1978. Acutely lethal levels of cadmium, copper, and zinc to adult male coho salmon and steelhead. Trans. Am Fish. Soc. 107:837-840.

D28 Chow, T.J., C.C. Patterson, and D. Settle. 1974. Occurrence of lead in tuna. Nature 251:159-161.

D29 Chow, T.J., C.B. Snyder, H. Snyder, and J.L. Earl. 1976. Lead content of some marine organisms. J. Environ. Sci. Health-Environ. Sci. Eng. 11:33-34.

D30 Courtois, L.A. 1976. Copper intoxication — A possible biochemical homologue. Bull. Environ. Contam. Toxicol. 15:205-210.

D31 Cousteau, J.Y. 1976. Time bomb in the Mediterranean. Saturday Review. Apr. 3:10-11.

D32 Cugurra, F. and G. Maura. 1976. Mercury content in several species of marine fish. Bull. Environ. Contam. Toxicol. 15:568-573.

D33 Davies, P.H., J.P. Goettl, J.R. Sinley and N.F. Smity. 1976. Acute and chronic toxicity of lead to rainbow trout Salmo gairdneri, in hard and soft water. Water Res. 10:199-206.

D34 DeGoeij, J.J.M., V.P. Guinn, D.R. Young and A.J. Mearns. 1974. Neutron activation analysis trace-element studies of Dover sole liver and marine sediments, p. 189-200. In Comparative studies of food and environmental contamination. International Atomic Energy Agency, Vienna.

No significant differences in trace elements were found in livers of Dover sole from polluted and unpolluted areas even though the sediments contained 10-150 times the concentration. Ranges of liver concentration (wet weight) were 0.050 to 0.39 ppm Hg, 0.23-1.9 ppm Se, 0.001-0.007 ppm Sb, 0.70-4.3 ppm As, 0.040-0.20 ppm Mo, 0.000-0.9 ppm Cd, 1.1-9.2 ppm Cu, 19-39 ppm Zn, 105-319 ppm Fe and 0.017-0.10 ppm Co.

D35 DeGoeij, J.J.M., J.L. Van Haaften, J.H. Koeman, P.S. Tjioe, and W.S.M. Van De Ven. 1975. Mercery and selenium in marine mammals and birds. Sci. Total Environ. 3:279-387.

D36 Fang, S.C. 1974. Uptake, distribution and fate of 203-Hg-ethylmercuric chloride in the guppy and the coontail. Environ. Res. 8:112-118.

D37 Fowler, B.A., D.A. Wolfe, and W.F. Hettler. 1975. Mercury and iron uptake by cytosomes in mantle epithelial cells of quahog clams (Mercanaria mercenaria) exposed to mercury. J. Fish. Res. Board Can. 32:1767-1775.

D38 Gilmartin, M. and N. Revelante. 1975. The concentration of mercury, copper, nickel, silver, cadmium, and lead in the northern Adriatic anchovy, Engraulis encrasicholus, and sardine, Sardina pilchardus. Fishery Bull. 73:193-202.

D39 Goettl. J.P. Jr. 1974. Acute toxicity of a zinc-polluted stream to four species of salmonids. Bull. Environ. Contam. Toxicol 12:464-469.

D40 Greig, R.A. 1975. Comparison of atomic absorbtion and neutron activaiton analyses for the determination of silver, chromium, and zinc in various marine organisms. Anal Chem. 47:1682-1684.

More variability of mean values of Ag, Cr, and Zn was due to sample preparation than to instrument variation. However statistical analysis of data showed agreement between methods for silver, but not for chromium or zinc. Thus the methods may not yet be accurate enough for precise environmental evaluation, but sufficient for monitoring distribution in a marine ecosystem.

D41 Grieg, R.A., A. Adams, and D.R. Wenzloff. 1977. Trace metal content of plankton and zooplankton collected from the New York Bight and Long Island Sound. Bull. Environ. Contam. Toxicol. 18:3-8.

Comparison of metal content of single species from different stations was difficult since different species were found at each station. Still the ranges of concentrations of Cu, Pb and Zn appear to be lower in Long Island Sound than in New York Bight.

D42 Greig, R.A. and R.A. McGrath. 1977. Trace metals in sediments of Raritan Bay. Mar. Pollut. Bull. 8:187-192.

The highest levels of metals found in Raritan Bay sediments were: Cd-15, Cr-260, Cu-1230, Ni-50, Pb-985, and Zn-815 ppm (dry weight). These high values were found in an area extending east from the mouths of Raritan River and Arthur Kill. The values are similar to those found in other polluted estuaries.

D43 Greig, R.A., B.A. Nelson, and D.A. Nelson. 1975. Trace metal content in the American oyster. Mar. Pollut. Bull. 6:72-73.

D44 Greig, R.A., D.R. Wenzloff, and J.B. Pearce. 1976. Distribution and abundance of heavy metals in finfish, invertebrates and sediments collected at a deepwater disposal site. Mar. Pollut. Bull. 7:185-187.

Muscle and liver tissues of fish collected at deepwater dumpsites contain less metals than fish from the continental shelf.

D45 Hartman, A.M. 1978. Mercury feeding schedules: Effects on accumulation, retention, and behavior in trout. Trans. Am. Fish. Soc. 107:369-375.

D46 Heisinger, J.F. and W. Green. 1975. Mercuric chloride uptake by eggs of the ricefish and resulting teratogenic effects. Bull. Environ. Contam. Toxicol. 14:665-673.

D47 Heterick, F.M., M.D. Knittel, and J.L. Fryer. 1979. Increased susceptibility of rainbow trout to infectious hematopoietic necrosis virus after exposure to copper. Appl. Environ. Microbiol. 37:198-201.

D48 Hildebrand, S.G., A.W. Andren, and J.W. Huckabee. 1975. Distribution and bioaccumulation of mercury in biotic and abiotic compartments of a contaminated river-reservoir system. Oak Ridge National Lab., Tenn. NTIS Report CONF-751058-1.

D49 Jan, T.K. and D.R. Young. 1978. Determination of microgram amounts of some transition metals in seawater by methyl isobutyl ketone-nitric acid successive extraction and flameless atomic absorbtion spectrophotometry. Anal. Chem. 50:1250-1253.

D50 Kelley, T.M., J.D. Jones and G.R. Smith. 1975. Historical changes in mercury contamination in Michigan walleyes (*Stizostedion vitreum vitreum*). J. Fish. Res. Board Can. 32:1745-1754.

D51 Kendall, M.W. 1975. Acute effects of methyl mercury toxicity in channel catfish (*Ictalurus punctatus*) kidney. Bull. Environ. Contam. Toxicol. 13:570-578.

D52 Kerfoot, W.B. and S.A. Jacobs. 1976. Cadmium accrual in combined wastewater treatment-aquaculture system. Environ. Sci. Technol. 10:662-667.

Clams and oysters accumulated more cadmium through ingestion of high-cadmium algae than through absorbtion from seawater. Safe levels of cadmium in seawater and effluent were estimated on the basis of retention of cadmium in algae, shellfish, and humans.

D53 Klaunig, J. 1975. Acute toxicity of a native mummichog population (*Fundulus heteroclitus*) to mercury. Bull. Environ. Contam. Toxicol. 14:534-536.

D54 Koirtyohann, S.R., R. Meers and L.K. Graham. 1974. Mercury levels in fishes from some Missouri lakes with and without known mercury pollution. Environ. Res. 8:1-10.

D55 Kramer, H.J. and B. Neidhart. 1975. The behavior of mercury in the system water-fish. Bull. Environ. Contam. Toxicol. 14:699-704.

D56 Laarman, P.W., W.A. Willford and J.R. Olson. 1976. Retention of mercury in the muscle of yellow perch (*Perca flavescens*) and rock bass (*Ambloplites rupestris*). Trans. Am. Fish. Soc. 105:296-300.

D57 MacKay, N.J., M.N. Kazako, R.J. Williams and M.I. Leedow. 1975. Selenium and heavy metals in black marlin. Mar. Pollut. Bull. 6:57-61.

D58 Matsumura, F., Y. Doherty, K. Furukawa, and G.M. Boush. 1975. Incorporation mechanisms *in vitro*. Environ. Res. 10:224-235.

D59 Matsunaga, K. 1975. Concentration of mercury by three species of fish from Japanese rivers. Nature 357:49-50.

D60 McDermott, D.J., G.V. Alexander, D.R. Young, and A.J. Mearns. 1976. Metal contamination of flatfish around a large submarine outfall. J. Water Pollut. Control Fed. 48:1913-1918.

D61 McDowell, R.A. 1973. Mercury concentrations in muscle tissues of carp collected from thirteen Wyoming habitats. National Marine Fisheries Service, Seattle. NTIS Report COM-75-10713/6ST.

D62 McLeese, D.W. 1974. Toxicity of copper at two temperatures and three salinities to the American lobster (*Homarus americanus*). J. Fish. Res. Board Can. 31:1949-1952.

D63 Mearns, A.J., P.S. Oshida, M.J. Sherwood, and D.R. Young. 1976. Chromium effects on coastal organisms. J. Water Pollut. Control Fed. 48:1929-1939.

D64 Mearns, A.J. and D.R. Young. 1976. Chromium in the coastal marine environment. NSF:IDOE Biological Effects Program, Texas A&M University, May 16-19.

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D66 Nehring, R. 1976. Aquatic insects as biological monitors of heavy metal pollution. Bull. Environ. Contam. Toxicol. 15:147-154.

D67 Nobbs, C.L. and D.W. Pearce. 1976. The economics of stock pollutants: The example of cadmium. Int. J. Environ. Stud. 8:245-255.

D68 O'Conner, D.V. and P.O. Fromm. 1975. The effect of methyl mercury on gill metabolism and blood parameters of rainbow trout. Bull. Environ. Contam. Toxicol. 13:406-411.

D69 Olson, G.F., D.I. Mount, V.M. Snarski, and T.W. Thorslund. 1975. Mercury residues in fathead minnows, *Pimephales promelas* Rafinesque, chronically exposed to methylmercury in water. Bull. Environ. Contam. Toxicol. 14:129-134.

D70 Olsson, M. 1976. Mercury level as a function of size and age in northrn pike, one and five years after the mercury ban in Sweden. Ambio 5:73-76.

D71 Pagenkopf, G.K., R.C. Russo, and R.V. Thurston. 1974. Effect of complexation on toxicity of copper to fishes. J. Fish. Res. Board Can. 31:462-465.

Computation of the concentration of different species of copper complexes in bioassay water of various hardness indicate that Cu^{2+} or $CuOH^{+}$ may be the toxic species.

D72 Patrick, F.M. and M. Loutit. 1976. Passage of metals in effluents, through bacteria to higher organisms. Water Res. 10:333-335.

D73 Pearce, J.B., J. Thomas, and R. Greig. 1975. May 1974 baseline investigation of deepwater dumpsite 106. U.S. Dept. of Commerce and Environmental Protection Agency: NOAA Dumpsite Evaluation Report 75-1.

The deep sea slickhead from deepwater dumpsite 106 had concentrations of metals several orders of magnitude greater than windowpane flounder from the N.Y. Bight. Otherwise, no differences in abundance and diversity of bottom fauna, metal content of invertebrates and finfish or metal content of sediments between the dumpsite and surrounding areas were evident.

D74 Pickering, Q.M. 1975. Chronic toxicity of nickel to fathead minnow (sic). J. Water Pollut. Control Fed. 46:760-765.

D75 Pipes, W.O. and E.D. Schaefer. 1973. Temperature and the toxicity of chromate and arsentae to the rotifer, *Philodina roseola*. Water Res. 7:1781-1790.

D76 Rehwoldt, R. and D. Karimian-Teherani. 1976. Uptake and effect of cadmium on zebrafish. Bull. Environ. Contam. Toxicol. 15:442-446.

D77 Rehwoldt, R., D. Karimian-Teherani and H. Altmann. 1976. Distribution of selected metals in tissue samples of carp, *Cyprinus carpio*. Bull. Environ. Contam. Toxicol. 15:734-737.

D78 Reimer, A.A. and R.D. Reimer. 1975. Total mercury in some fish and shellfish along the Mexican coast. Bull. Environ. Contam. Toxicol. 14:105-111.

D79 Renzoni, A. and E. Bacci. 1976. Bodily distribution accumulation, and excretion of mercury in a fresh-water mussel. Bull. Environ. Contam Toxicol. 15:366-373.

D80 Richin, R.T. and A.C. Risser. 1975. Total mercury in water, sediment and selected aquatic organisms, Carson River, Nevada —1972. Pestic. Monit. J. 9:44-54.

D81 Roales, R.R. and A. Pearlmutter. 1974. Toxicity of zinc and cygon, applied singly and jointly, to zebrafish embryos. Bull. Environ. Contam. Toxicol. 12:475-480.

D82 Roesijadi, G., S.R. Petrocelli, J.W. Anderson, J.B. Presley, and R. Sims. 1974. Survival and chloride ion regulation of the porcelain crab, *Petrolisthes armatus*, exposed to mercury. Mar. Biol. 27:213-217.

D83 Romeril, M.G. and M.H. Davis 1976. Trace metal levels in eels grown in power station cooling water. Aquaculture. 8:139-149.

D84 Rose, C.L. and D.P. Middaugh. 1974. Retention of two mercuricals by striped mullet. Water Res. 8:173-177.

D85 Schultz, C.D., D.Crear, J.E. Pearson, J.B. Rivers, and J.W. Hylin. 1976. Total and organic mercury in the Pacific blue marlin. Bull. Environ. Contam. Toxicol. 15:230-234.

D86 Sellers, C.M., A.G. Heath, and M.L. Bass. 1975. The effect of sublethal concentrations of copper and zinc on ventilatory activity, blood oxygen, and pH in rainbow trout (*Salmo gairdneri*). Water Res. 9:401-408.

D87 Shin, E.and P.A. Krenkel. 1976. Mercury uptake by fish and biomethylation mechanisms. J. Water Pollut. Control Fed. 48:473-501.

D88 Shomura, R.S. and W.L. Craig. 1972. Mercury in several species of billfishes taken off Hawaii and southern California. Presented at International Billfish Symposium, Hawaii. 2:160-163.

D89 Shore, R., G. Carney, and T. Stygall. 1975. Cadmium levels and carbohydrate metabolism in limpets. Mar. Pollut. Bull. 6:187-189.

D90 Smith, B.P., E. Hejtmancik, and B.J. Camp. 1976. Acute effects of cadmium on *Ictalurus punctatus* (catfish). Bull. Environ. Contam. Toxicol 15:271-277.

D91 Smith, E.C., F. Berkes, and J.A. Spence. 1975. Mercury levels in fish in the La Grande River area, Northern Quebec. Bull. Environ. Contam. Toxicol. 13:673-677.

D92 Sorensen, E.M. 1976. Thermal effects on the accumulation of arsenic in green sunfish, *Lepomis cyanellus*. Arch. Environ. Contam. Toxicol. 4:8-17.

D93 Spehar, R.L., E.N. Leonard, and D.L. DeFoe. 1978. Chronic effects of cadmium and zinc mixtures of flagfish (*Jordanella floridae*). Trans. Am. Fish. Soc. 107:354-360.

D94 Suzuki, T., M. Tomoya, and T. Chiharu. 1973. Distribution and form of mercury in marine fish. Bull. Environ. Contam. Toxicol. 10:347-355.

The bodily distribution pattern of mercury was found to differ according to the chemical form of mercury ingested. Biotransformation of mercury was considered possible but not substantiated.

D95 Tsai, S., G. Boush, and F. Matsumura. 1975. Importance of water pH in accumulation of inorganic mercury in fish. Bull. Environ. Contam. Toxicol. 13:188-193.

D96 Updegraf, K.F. and J.L. Sykor. 1976. Avoidance of lime-neutralized iron hydroxide solutions by coho salmon in the laboratory. Environ. Sci. Technicol. 10:51-54.

D97 VanMeter, W.P. 1974. Heavy metal concentration in fish tissue of the upper Clark Fork River. NTIS Report PB-237-429.

D98 Varanasi, U., P.A. Robisch, and D.C. Malins. 1975. Structural alterations in fish epidermal mucus produced by water-borne lead and mercury. Nature. 258:431-432.

D99 Voyer, R.A. 1975. Effect of dissolved oxygen on the acute toxicity of cadmium to the mummichog, *Fundulus* heteroclitus (L), at various salinities. Trans. Am. Fish. Soc. 104:129-134.

D100 Voyer, R.A., P.P. Yevich, and C.A. Barszcz. 1975. Histological and toxicological responses of the mummichog, *Fundulus heteroclitus* (L) to combinations of levels of cadmium and dissolved oxygen in a freshwater medium. Water Res. 9:1069-1074.

Toxicity of cadmium increased as DO decreased: 2.9 mg/l Cd produced only 45% mortality in 96 hours at 8.5 mg/l DO while the same Cd concentration produced 80% mortality in 48 hours at 2.3 mg/l DO. No histopathology was evident at these low concentrations. Mummichogs are one order of magnitude more resistant to Cd in seawater than in freshwater.

D101 Weiss, J.S. 1976. Effects of mercury cadmium, and lead salts on regeneration and ecdysis in the fiddler crab, *Uca pugilator*. Fishery Bull. 74:464-467.

D102 Winner, R.W. 1976 Toxicity of copper to daphnids in reconstituted and natural waters. EPA/600/3-76-051. NTIS Report PB-252 915/4ST.

D103 Wobeser, G. 1975. Exposure of methyl mercury chloride and mercuric chloride to rainbow trout (*Salmo gairdneri*) fry and fingerlings. J. Fish. Res. Board Can. 32:2005-2022.

D104 Wright, D.A. 1976. Heavy metals in animals from the North East Coast Mar. Pollut. Bull. 7:36-38.

D105 Yamanaka, S. and K. Ueda. 1975. High ethylmercury in river fish by man-made pollution. Bull. Environ. Contam. Toxicol. 14:409-414.

D106 Zitko, V. 1975. Toxicity and pollution potential of thallium. Sci. Total Environ. 4:185-192.

D107 Zitko, V., W.V. Canson, and W.G. Carson. 1975. Thallium occurrence in the environment and toxicity to fish. Bull. Environ. Contam. Toxicol. 13:23-30.

E NITROGENOUS COMPOUNDS

E1 Bower,C.E. and J.P. Bidwell. 1978. Ionization of ammonia in seawater: effects of temperature, pH, and salinity. J. Fish. Res. Board Can. 35:1012-1016.

Tables show the percent of unionized ammonia present in seawater depending on pH and temperature for four ranges of salinity; 18-22%, 23-27%, 28-31%, and 32-40%.

E2 Colt, J.and G. Tchobanoglous. 1976. Evaluation of the short-term toxicity of nitrogenous compounds to channel catfish, *Ictalurus punctatus*. Aquaculture 8(3):209-224.

The acute toxicity of unionized ammonia, nitrite, nitrous acid, and nitrate at 30° were (96 hours LD_{50}'s) 3.8, 44, 0.0002-0.0003, and 6,200 mg/l, respectively in static systems. The formulae for conversion of total ammonia and nitrite into their unionized forms (NH_3 and HNO_2) are derived.

E3 Downing, K.M. and J.C. Merkens. 1955. The influence of dissolved-oxygen concentration on the toxicity of unionized ammonia to rainbow trout (*Salmo gairdnerii* Richardson). Ann. Appl. Biol. 43:243-246.

Survival time of trout in a flow through system containint 0.86 to 1.96 ppm unionized ammonia nitrogen increased as dissolved oxygen increased from 1.5 to 8.5 ppm.

E4 Emerson, K., R.C. Russo, R.E. Lund, and R.V. Thurston. 1975. Aqueous ammonia equilibrium calculations: effect of pH and temperature. J. Fish. Res. Board Can. 32:2379-2383.

Presents tables and a formula for calculation of the amounts of the amount of unionized (aqueous) ammonia in fresh water at a given pH and temperature.

E5 Hale, S.S. 1975. The role of benthic communities in the nitrogen and phosphorus cycles of an estuary, p. 291-308. *In* Howell, F.G., J.B. Gentry and M.H. Smith (eds.), Mineral cycling in southeastern ecosystems. ERDA Symposium Series 1975. (CONF-740513). Univ. of Rhode Island Marine Reprint No. 57.

Quantitative measurement of the nutrient regeneration role of the benthos by making *in-situ* measurements of the net sediment-water flux of nutrients.

E6 Haywood, J.E. and A.L. Kuhn. 1970. A colorimetric method for NH_3 in natural waters. Water Res. 4:805-811.

E7 Hazel, C.R., W. Thomsen, and S.J. Meith. 1971. Sensitivity of striped bass and stickleback to ammonia in relation to temperature and salinity. Calif. Fish Game 57:154-161.

Ninety six hour TL_m of striped bass for unionized NH_4OH were 2.8 mg/l in freshwater, 2.8 mg/l in one third seawater, and 2.0 mg/l in seawater at 15°C. These values for sticklebacks were 2.1, 5.2, and 10.4, respectively. Values at 23° were also determined. Relationships of unionized ammonia to salinity, temperature, and pH do not agree with Bower, 1978.

E8 Inman, R.C. 1974. Acute toxicity of Phos-Chek (trade name) 202 and diammonium phosphate to fathead minnows. NTIS Report AD/A-006-122.

E9 Konikoff, Mark. 1975. Toxicity of nitrite to channel catfish. Prog. Fish-Cult. 37:96-98.

The 24, 48, 72 and 96 hour TL_m's of channel catfish to nitrite at 21°C were 33.8, 28.8, 27.3, and 24.8 mg/l respectively, as determined by static bioassays. Nitrite toxicity can be detected in fish by examination of blood for methemoglobinemia. Test water pH was not mentioned.

E10 Milne, M.D., B.H. Scribner, and M.A. Crawford. 1958. Non-ionic diffusion and the excretion of weak acids and bases. Am. J. Med. 24:709-729.

Intricate chemistry of non-ionic diffusion of ammonia through biological membranes.

E11 Robinson-Wilson, E.F. and W.K. Seim. 1975. The lethal and sublethal effects of a zirconium process effluent on juvenile salmonids. Water Res. Bull. 11:975-986.

E12 Rucker, R.R. and P.M. Kangas. 1974. Effect of nitrogen supersaturated water on coho and chinook salmon. Prog. Fish-Cult. 36:152-156.

E13 Russo, R.C., C.E. Smith and R.V. Thurston. 1974. Acute toxicity of nitrite to rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 31:1653-1655.

Flow through bioassays using rainbow trout of four different sizes showed 96 hour LD_{50} values ranging from 0.19 mg/l NO_2-N to 0.39 mg/l.

E14 Russo, R.C. and R.V. Thurston. 1977. The acute toxicity of nitrite to fishes, p. 118-131. *In* R.A. Tubb (ed.). Recent advances in fish toxicology. EPA Ecol. Res. Ser. EPA-600/3-77-085, Corvallis, Oregon.

The average 96 hour LC_{50} was 0.24 mg/l of $N)_2$-N. Toxicity was independant of pH within the range of 7.5-8.5 and was correlated with NO_2^- and not with HNO_2. An increase in chlorine concentration caused a decrease in nitrite toxicity.

E15 Seligson. D. and H. Seligson. 1951. A microdiffusion method for the determination of nitrogen liberated as ammonia. J. Lab. Clin. Med. 38:324-330.

A simple diffusion cell and a mechanical rotator are used to measure total ammonia nitrogen by Nesslerization in amounts from 2 to 200 ug of nitrogen in blood and urine.

E16 Smith, C.E. and R.C. Russo. 1975. Nitrite-induced methemoglobinemia in rainbow trout. Prog. Fish-Cult. 37:150-152.

An elevation in trout methemoglobin occurred after exposure to as little as 0.096 mg/l NO_2.

E17 Thurston, R.V., R.C. Russo and C.E. Smith. 1978. Acute toxicity of ammonia and nitrite to cutthroat trout fry. Trans. Am. Fish. Soc. 107:361-368.

Flow through bioassays resulted in 96 hour LC$_{50}$ values of unionized ammonia from 0.5 to 0.8 mg/l while 36 day LC$_{50}$'s were 0.3-0.6 mg/l. Nitrite LC$_{50}$ values were 0.5-0.6 mg/l NO$_2$-N for 96 hours and 0.4 mg/l for 36 days. Gill, liver and kidney tissues of fry exposed to 0.34 mg/l unionized ammonia for 29 days showed degenerative changes.

F OIL AND PETROCHEMICALS

F1 Anderson, J.W., J.M. Neff, and S.R. Petrocelli. 1974. Sublethal effects of oil, heavy methals and PCBs on marine organisms. Texas A & M University. NTIS Report PB-238 514.

F2 Anderson, R.D. and J.W. Anderson. 1976. Oil bioassays with the American oyster, *Crassostrea virginica* (Gmelin). Proc. Natl. Shellfish. Assoc. 65:38-42.

F3 Becker,C.D., J.A. Lichatowich, M.J. Schneider and J.A. Strand. 1973. Regional survey of marine biota for bioassay standardization of oil and oil dispersant chemicals. American Petroleum Institute.. Pub. 4167.

F4 Brenniman, G., R. Hartung, and W.J. Weber. 1976. A continuous flow bioassay method to evaluate the effects of outboard motor exhausts and selected aromatic toxicants on fish. Water Res. 10:165-169.

F5 Clark, R.C. and J.S. Finley. 1974. Tidal aquarium for laboratory studies of environmental effects on marine organisms. Prog. Fish-Cult. 36:134-137.

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F20 Morrow, J.E. 1974. Effects of crude oil and some of its components on young coho and sockeye salmon. EPA Report 660/3-73-018.

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G PATHOLOGY AND HISTOLOGY

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G3 Armstrong, D.A. and R.E. Millemann. 1974. Pathology of acute poisoning with the insecticide sevin in the bent-nosed clam, *Macoma nasuta*. J. Invertebr. Pathol. 24:201-212.

Sevin concentrations of 15-30 mg/l resulted in epithelial necrosis, primarily of gill, mantle, siphon and suprabranchial gland.

G4 Cairns, M.A. and A.R. Christian. 1978. Effects of hemorrhagic stress on several blood parameters in adult rainbow trout (*Salmo gairdneri*). Trans. Am. Fish. Soc. 107:334-340.

G5 Clary, J.R. and S.D. Clary. 1978. Swim bladder stress syndrome. Salmonid. March/April:8-9.

Describes four distinct stages of SBSS, which has similar symptoms to gase bubble disease but is usually due to low DO concentrations over a 5 hour period. Adrenal exhaustion results in insufficient catecholamine production which prevents reabsorbtion of excess gas from the swim bladder.

G6 Cook, D.W. and S.W. Lofton. 1975. Pathogenicity studies with a *Streptococcus* isolated from fishes in an Alabama-Florida fish kill. Trans. Am. Fish. Soc. 104:286-288.

G7 Dively, J.E., W.H. Neff, and A. Anthony. 1977. Blood P_{O2}, P_{CO2}, and pH changes in brook trout (*Salvelinus fontinalis*) exposed to sublethal levels of acidity. Comp. Biochem. Physiol. 57A:347-351.

Exposure of brook trout to near toxic levels of acid resulted in elevated P_{CO2}, hematocrit, and ventilation rate. P_{O2} and pH remained constant in spite of mucification of gills and skin.

G8 Dolsky, M.F. and A. Anthony. 1973. Histochemical analysis of prolactin and Stannius corpuscle cells of brook trout exposed to low pH. Proc. Pa. Acad. Sci. 47:123-125.

Cytopathological and cytophotometric studies of pituitary prolactin cells and Stannius corpuscle cells of acid-exposed fish show increased levels of RNA and protein after 3 days, coinciding with skin mucification. This suggested an osmoregulatory function.

G9 Dukes, T.W. and A.R. Lawler. 1975. The ocular lesions of naturally occurring limphocystis in fish. Can. J. Comp. Med. 39:406-410.

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Histological changes in liver, kidney, and skin of carp with hemorrhagic septicemia were variable and non-specific. However, eosinophilic cytoplasmic inclusions in the isthmal ganglion of the brain proved diagnostic for red rot.

G11 Fontaine, C.T. and D.V. Lightner. 1972. Observations on the process of wound repair in penaeid shrimp. J. Invertebr. Pathol. 22:23-33.

G12 Fromm, P.O. and K.R. Olson 1973. Industrial and municipal wastes: Action of some water soluble pollutants on fish. Completion report: Office of Water Research and Technology. NTIS Report PB 237 428.

The effects of mercurials on gill epithelium ultrastructure are examined by SEM, TEM, and electron microprobe analysis. Changes in the lamellar surface and in chloride cells are seen with these techniques that are not seen with light microscopy.

G13 Gardner, G.R. and P.P. Yevich. 1970. Histological and hematological responses of an estuarine teleost to cadmium. J. Fish. Res. Board Can. 27:2185-2199.

Histopathological changes in intestinal tract of _Fundulus_ appeared 1 hour after exposure to 50 ppm cadmium, in kidney after 12 hours, and in gills after 20 hours. Blood eosinophils increased 45%, although those in the head kidney were relatively immature.

G14 Glantz, P.J. and G.E. Krantz. 1965. _Escherichia coli_ serotypes isolated from fish and their environment. Health Lab. Sci. 2:54-63.

Serological studies provided information on the exchange of _E. coli_ between the aquatic environment, fish, domestic animals and man. Trout fed serotypes pathogenic to man and animals did not develop disease symptoms, but the bacteria were able to survive in their intestines.

G15 Gopalan, U.K. and J.S. Young 1975. Incidence of shell disease in shrimp in the New York Bight. Mar. Pollut. Bull. 6:149-153.

Chitinoclastic bacteria were thought to be the primary cause of this exoskeleton erosion since tetracycline prevented its onset. These bacteria abound where the natural water is rich in organics, especially cellulose, as in the New York Bight.

G16 Jeffries, H.P. 1971. Chemical responses by marine organisms to stress. Stress in hard clams from a polluted estuary. EPA-R3-72-017. NTIS Report PB-213 193.

The ratio of free taurine to glycine in mantle and gill tissue of the clam *Mercenaria mercenaria* is diagnostic for a generalized stress syndrome preceding mass mortality. Possibly as the alpha-amino acids are depleted, taurine increases to maintain osmotic balance.

G17 Krantz, G.E., L.R. Buchanan, C.A. Farley, and H.A. Carr. 1972. *Minchinia nelsoni* in oysters from Massachusetts waters. Proc. Natl. Shellfish. Assoc. 62:83-85.

G18 Lightner, D.V. and D.H. Lewis. 1975. A septicemic bacterial disease syndrome of penaeid shrimp. Mar. Fish. Rev. 37:25-28.

G19 Mahoney, J.B., F.H. Midlige, and D.G. Deuel. 1973. A fin rot disease of marine and euryhaline fishes in the New York Bight. Trans. Am. Fish. Soc. 102:596-605.

Bacteria of the genera *Aeromonas*, *Vibrio* and *Pseudomonas* were thought to be responsible for annual epizootics of fin rot.

G20 McLeay, D.J. 1975. Sensitivity of blood cell counts in juvenile coho salmon (*Oncorhynchus kisutch*) to stressors including sublethal concentrations of pulpmill effluent and zinc. J. Fish. Res. Board. Can. 32:2357-2364.

G21 Miller, W.C. and R. Toon. 1973. *Mycobacterium marinum* in gulf fishermen. Arch. of Environ. Health 27:8-10.

G22 Mix, M.C. 1975. Neoplastic disease of Yaquina Bay bivalve mollusks. Proceedings of the Annual Hanford Biology Symposium (13th). 1:369-386.

G23 Mudge, J.E., J.L. Dively, W.H. Neff, and A. Anthony. 1976. Interrenal histochemistry of acid-exposed brook trout, *Salvelinus fontinalis* (Mitchill). Gen. Comp. Endocrinol. 31:208-215.

"The findings support the conclusion that there is a marked inhibition of RNA synthesis and presumably of steroidogenesis in interrenal tissue following acute exposure of trout to low pH."

G24 Notter, M.F.D., J.E. Mudge, W.H. Neff, and A. Anthony. 1976. Cytophotometric analysis of RNA changes in prolactin and stannius corpuscle cells of acid-stressed brook trout. Gen. Comp. Endocrinol. 30:273-284.

"The overall findings are in accord with the concept that these two tissues have independent but synergistic roles in osmoregulation during acclimation to acid stress."

G25 Pauley, G.B. 1975. Introductory remarks on diseases of crustaceans. Mar. Fish. Rev. 37:2-3.

G26 Pauley, G.B., M.W. Newman, and E. Gould. 1975. Serum changes in the blue crab, *Callinectes sapidus*, associated with *Paramoeba perniciosa*, the causative agent of gray crab disease. Mar. Fish. Rev. 37:34-38.

G27 Pendleton, D. 1975. Fish tumors: carcinogenic indicators? Sci. News. 107:157-158.

G28 Perkins, F.O. 1975. Fine structure of *Minchinia sp.* (haplosporida) sporulation in the mud crab, *Panopeus herbstii*. Mar. Fish. Rev. 37:46-60.

G29 Plumb.J.A. 1972. Some biological aspects of Channel Catfish Virus Disease. NOAA-72091907. NTIS Report COM-72-11301.

G30 Plumb,J.A., J.H. Schachte, W.A. Rogers, J.L. Gaines, and R.P. Phelps. 1976. Parasites and diseases of cultured catfish. NOAA-76021701. NTIS Report PB-252 360/3ST.

G31 Quick, J.A. Jr. and G.E. Henderson. 1974. Effects of *Gymnodinium breve* red tide on fishes and birds: A preliminary report on behavior, anatomy, hematology and histopathology. Proceedings of Gulf Coast Regional Symposium on Diseases of Aquatic Animals, Baton Rouge, La. 85-114.

G32 Sandifer, P.A. and P.J. Eldridge. 1974. Observations on the incidence of shell disease in South Carolina blue crabs, *Callinectes sapidus* (Rathbun). NOAA-75053003-14. Proceedings of Gulf Coast Regional Symposium on Diseases on Aquatic Animals, Baton Rouge, La. 161-184.

G33 Sherwood, M.J. and B.B. McCain. 1976. Comparison of fin erosion disease: Los Angeles and Seattle, p.143-147. *In* Southern Calif. Coastal Water Research Project - Annual Rept., El Segundo, Calif.

A high PCB content was found in the tissues of both Dover sole and starry flounder with fin erosion, while no similar prevalence of DDT or selected metals occurred in both species. Bacterial etiology was ruled out because of lack of inflammatory response and paucity of marine pathogens at the site of the lesions.

G34 Silbergeld, E.K. 1974. Blood glucose: A sensitive indicator of environmental stress in fish. Bull. Environ. Contam. Toxicol. 11:20-25.

Blood glucose levels in johnny darters were significantly elevated a few days after exposure to 2.33 μg/l dieldrin (one fourth of the LD_{50}) and also after confinement in laboratory conditions.

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Superficial examination of shoreline fish and invertebrates for gross pathology, deciding that there is a relativley high natural incidence of pathology in marine animals from unpolluted areas.

G36 Smith, A.C., R.L. Taylor, H. Chun-Akana, and F. Ramos. 1973. Intestinal tumor in the sea cucumber, *Holothuria leucospilata*. J. Invertebr. Pathol. 22:305-307.

G37 Sparks, A.K. and C.T. Fontaine. 1973. Host response in the white shrimp, *Penaeus setiferus*, to infection by the larval trypanorhynchid cestode, *Prochristianella penaei*. J. Invertebr. Pathol. 22:213-219.

G38 Stagner, J.I. and J.R. Redmond. 1975. The immunological mechanisms of the horseshoe crab, *Limulus polyphemus*. Mar. Fish. Rev. 37: 11-19.

G39 Steinhaus, E.A. and M.E. Martignoni. 1970. An abridged glossary of terms used in invertebrate pathology. Pacific Northwest Forest and Range Experiment Station. U.S. Dept. Agricul. Forest Service.

G40 Udey, L.R. 1978. Effects of temperature on the infectious process in Pacific salmonids, p. 103-106. *In* Gerhold, R.M. (ed). Proceedings of the Symposium: Microbiology of power plant thermal effluents. The University of Iowa Press, Iowa City, Iowa.

In general, the higher the water temperature, the shorter the survival time of salmonids, although each of the six infectious agents assayed had a different pattern of mortality depending on factors such as optimum growth temperature and fish species.

G41 Umphlett, C.J. and E.M. McCray, Jr. 1975. A brief review of the involvements of *Lagenidium*, an aquatic fungus parasite, with arthropods. Mar. Fish. Rev. 37:61-64.

G42 Wedemeyer, G. 1973. Some physiological aspects of sublethal heat stress in juvenile steelhead trout (*Salmo gairdneri*) and coho salmon *Oncorhynchus kisutch*). J. Fish. Res. board Can. 30:831-834.

G43 Wharton, J.H., R.D. Ellender, and P.K. Stocks. 1974. *In vitro* cultivation of cells from the silver perch, *Bairdiella chrysura*: A substrate for lymphocystis replication. NOAA-75053003-12. Proceedings of Gulf Coast Regional Symposium in Diseases of Aquatic Animals, Baton Rouge, La.

G44 Wolke, R.E. and R.A. Murchelano. 1976. A case report of an epidermal papilloma in *Mustelus canis*. J. Wildlife Dis. 12:167-171.

Although virus particles were lacking in T.E.M. examination, the histological appearance of this neoplasm was similar to fish pox in European cyprinids. The regressive, pleomorphic and dysplasic cells would indicate carcinoma *in situ*.

G45 Young, J.S. and J.B. Pearce. 1975. Shell disease in crabs and lobsters from New York Bight. Mar. Pollut. Bull. 6:101-105.

Chitinoclastic bacteria are thought to be responsible for exoskeletal erosion of crustaceans from sewage sludge dumping areas. The disease can be reproduced in the lab within 6 weeks by exposing animals to sewage contaminated sediments.

G46 Ziskowski, J. and R. Murchelano. 1975. Fin erosion in winter flounder. Mar. Pollut. Bull. 6:26-29.

Progressive fin erosion of demersal flatfish occurred significantly more in the New York Bight than adjacent areas. Infrequent occurrence of inflammatory response and absence of massive bacteriological infection suggest that no primary pathogen is responsible. Rather, necrosis of fin epitherlium may result from stress induced by metal toxicity of the sediments and associated low DO.

H PESTICIDES, PCB's AND OTHER ORGANIC TOXICANTS

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H3 Adelman, I.R., L.L. Smith, and G.D. Siesennop. 1976. Chronic toxicity of guthion to the fathead minnow, (*Pimephales promelas* Rafinesque). Bull. Environ. Contam. Toxicol. 15:726-733.

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H6 Anon. 1974. The national pesticide monitoring program. National Agricultural Chemicals Assn. Report.

H7 Anon. 1975. South Dakota toxaphene use study June-September 1975. EPA Report 330/2-75-007.

H8 Anon. 1976. Fishing is halted after PCB's found. New York Times. Sept. 27:23.

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H10 Armstrong, D.A. and R.E. Millemann. 1974. Pathology of acute poisoning with the insecticide sevin in the bent-nosed clam. *Macoma nasuta*. J. Invertebr. Pathol. 24:201-212.

H11 Armstrong, D.A. and R.E. Millemann. 1974. Effects of the insecticide sevin and its first hydrolytic product, 1-naphthol, on some early devleopmental stages of the bay mussel, *Mytilus edulis*. Mar. Biol. 28:11-15.

H12 Bevenue, A., J.N. Ogata, L.S. Tengan, and J.W. Hylin. 1975. Mirex residues in wildlife and soils, Hawaiian pineapple growing areas-1972-1974. Pestic. Monit. J. 9:141-149.

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H16 Boyle, R.H. 1975. Of PCB ppms from GE and a snafu from EPA and DEC. Audubon. 77:127-133.

H17 Branson, D.R., G.E. Blau, H.C. Alexander, and W.B. Neely. 1975. Bioconcentration of 2,2 '-tetrachlorobiphenyl in rainbow trout as measured by an accelerated test. Trans. Am. Fish. Soc. 104:785-892.

H18 Brown,J.R., L. Chow, and F. Chai. 1975. Distribution of organochlorine pesticides in an agricultural environment Holland Marsh, Ontario-1970-72. Pestic. Monit. J. 9:30-33.

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H31 Desaiah, D. and R.B. Koch. 1975. Inhibition of ATP pases activity in channel catfish and its reduction product. Bull. Environ. Contam. Toxicol. 13:153-158.

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H42 Hamelink, J.L. and R.C. Waybrant. 1976. DDE and lindane in a large-scale model lentic ecosystem. Trans. Am. Fish. Soc. 105:124-134.

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H45 Hattula, M. 1974. Some aspects of the recovery of chlorinated residues (DDT-type compounds and PCB) from fish tissue by using different extraction methods. Bull. Environ. Contam. Toxicol. 12:301-307.

H46 Heesen, T.C. and D.R. Young. 1977. Halogenated hydrocarbons in wastewaters: Knows and unknowns, p. 33-38. *In* Southern Calif. Coastal Water Research Project - Annual Rept., El Segundo, Calif.

The DDT's and PCB's currently monitored by coastal dischargers in California are an order of magnitude below the level of total chlorinated benzenes in municipal wastes and these, in turn, are an order of magnitude below the level of low-molecular weight halogenated hydrocarbons.

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H48 Ivie, G., J.R. Gibson, H.E. Bryant, J.J. Begin, J.R. Barnet, and H. Dorough. 1974. Accumulation, distribution and excretion of mirex-14C in animals exposed for long periods to the insecticide in the diet. J. Agric. Food Chem. -ACS. 22:646-653.

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H52 Khan, H.M. and M.A. Kahn. 1974. Biological magnification of photodieldrin by food chain organisms. Arch. Environ. Contam. Toxicol. 2:289-301.

H53 Khan, H.M., S. Neudorf and M.A. Khan. 1975. Absorption and elimination of photodieldrin by daphnia and goldfish. Bull. Environ. Contam. Toxicol. 13:582-587.

H54 Klaassen, H.E. and A.M. Kadoum. 1975. Insecticide residues in the Tuttle Creek reservoir ecosystem, Kansas-1970-71. Pest. Monit. J. 9:89-93.

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H56 Koeing, C.C., R.J. Livingston and C.R. Cripe. 1976. Blue crab mortality: interaction of temperature and DDT residues. Arch. Environ. Contam. Toxicol. 4:119-128.

H57 Korn, S. and E. Russell. 1974. Acute toxicity of twenty insecticides to striped bass, Morone saxatillis. Calif. Fish Game 60:128-131.

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H59 Kpekata, A.E. 1975. Polychlorinated biphenlys (PCB's) in the Rivers Avon and Frome. Bull. Environ. Contam. Toxicol. 14:687-692.

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H61 Lee, J.H., J.R. Sylvester, and C.E. Nash. 1975. Effects of mirex and methoxychlor on juvenile and adult sttiped mullet. Mugil cephalus L. Bull. Environ. Contam. Toxicol. 14:180-186.

H62 Lefftler, C.W. 1975. Effects of ingested mirex and DDT on juvenile Callinectes sapidus Rathbun. Environ. Pollut. 8:293-310.

H63 Lieb, A.J., D.D. Bills, and R.O. Sinnhuber. 1974. Accumulation of dietary polychlorinated biphenyls (aroclor 1254) by rainbow trout. J. Agric. Food Chem. 22:638-642.

H64 Lincer, J.L., M.E. Haynes, and M.L. Klein. 1976. The ecological impact of synthetic organic compoudns on estuarine ecosystems. EPA/600/3-76/075. NTIS Report PB-259 943/9ST.

H65 Macek, K.J. 1975. Acute toxicity of pesticide mixtures to bluegills. Bull. Environ. Contam. Toxicol. 14:648-653.

H66 Macek, K.J., K.S. Buxton, S. Sauter, S. Gnilka, and J.W. Dean. 1976. Chronic toxicity of atrazine to selected aquatic invertebrates and fishes. EPA/600/3-76/047. NTIS Report PB-255 439/2ST.

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H68 Marking, L.L. and W.I. Mauck. 1975. Toxicity of paired mixtures of Candidate Forest insecticides to rainbow trout. Bull. Environ. Contam. Toxicol. 13:518-523.

H69 Mauck, W.L., F.L. Mayer, and D.D. Holz. 1976. Simazine residue dynamics in small ponds. Bull. Environ. Contam. Toxicol. 16:1-7.

H70 Mauck, W.L., L.E. Olson, and L.L. Marking. 1976. Toxicity of natural pyrethrins and five pyrethroids to fish. Arch. Environ. Contam. Toxicol. 16:1-7.

H71 Mayer, F.L., P.M. Mehrle, and P.L. Crutcher. 1978. Interactions of toxaphene and vitamin C in channel catfish. Trans. Am. fish. Soc. 107:326-333.

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H73 Mehrle, P.M., W. Johnson, and F.L. Mayer. 1974. Nutritional effects on chlordane toxicity in rainbow trout. Bull. Environ. Contam. Toxicol. 12:513-517.

H74 Mehrle, P.M. and F.L. Mayer. 1975. Toxaphene effects on growth and bone composition of fathead minnows, Pimephales promelas. J. Fish. Res. Board Can. 32:593-598.

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H79 Peterson, J.E., K.M. Stahl, and D.L. Meeker. 1976. Simplified extraction and cleanup for determining organochlorine pesticides in small biological samples. Bull. Environ. Contam. Toxicol. 15:135-139.

H80 Petrocelli, S.R., J.W. Anderson, and A.R. Hanks. 1975. Biomagnification of dieldrin residues by food-chain transfer from clams to blue crabs under controlled conditions. Bull. Environ. Contam. Toxicol. 13:108-116.

H81 Pfuderer, P. and A.A. Francis. 1975. Phthalate esters: Heartrate depressors in the goldfish. Bull. Environ. Contam. Toxicol. 13:275-279.

H82 Pfuderer, P., S. Janzen, and W.T. Rainey. 1975. The identification of phthalic acid esters in the tissues of cyprinodont fish and their activity as heartrate depressors. Environ. Res. 9:215-223.

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H84 Redmann, G. 1973. Studies on the toxicity of mirex to the estuarine grass shrimp, *Palaemonetes pugio*. Gulf Res. Rep. 4:272-277.

H85 Reimold, R.J. and M.H. Shealy. 1976. Chlorinated hydrocarbon pesticides and mercury in coastal young-of-the-year finfish, South Carolina and Georgia-1972-1974. Pestic. Monit. J. 9:170-175.

H86 Rensberger, B. 1975. Pesticide linked to defects in fish. New York Times. Apr. 10:8.

H87 Roberts, D. 1975. Sub-lethal effects of chlorinated hydrocarbons on bivalves. Mar. Pollut. Bull. 6:20-24.

H88 Roesijadi, G., S.R. Petrocelli, J.W. Anderson, C.S. Giam, and G.E. Neff. 1976. Toxicity of polychlorinated byphenyls (aroclor 1254) to adult, juvenile, and larval stages of the shrimp *Palaemonetes pugio*. Bull. Environ. Contam. Toxicol. 15:297-304.

H89 Rosenberg, R., O. Grahn, and L. Johansson. 1975. Toxic effects of aliphatic chlorinated by-products from vinyl chloride production on marine animals. Water. Res. 9:607-612.

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H91 Scales, E.H. and J.D. Yarbrough. 1975. Endrin uptake in insecticide-resistant and susceptible mosquitofish (*Gambusia affinis*). J. Agric. Food Chem. 23:1076-1079.

H92 Schaper, R.A. and L.A. Crowder. 19[illegible] Uptake of 36Cl-toxaphene in mosquito fish, *Gambusia affinia*. Bull. Environ. Contam. Toxicol. 15:581-587.

H93 Shaw, B. and P.K. Hopke. 1975. The dynamics of diquat in a model ecosystem. Environ. Lett. 8:325-335.

H94 Sims, G.G., C.E. Cosham, J.R. Campbell, and M.C. Murray. 1975. DDT residues in cod livers from the maritime provences of Canada. Bull. Environ. Contam. Toxicol. 14:505-512.

H95 Spacie, A. 1976. Acute and chronic parathion toxicity to fish and invertebrates. EPA-18050-HQG. NTIS Report PB-257 800/3ST.

H96 Sugawara, N. 1974. Effect of phthalate esters on Shrimp. Bull. Environ. Contam. Toxicol. 12:421-424.

H97 Tagatz, M.E., P.W. Borthwick, and J. Forester. 1975. Seasonal effects of leached mirex on selected estuarine animals. Arch. Environ. Contam. Toxicol. 3:371-383.

H98 Taruski, A.G., C.E. Olney, and H.E. Winn. 1975. Chlorinated hydrocarbons in cetaceans. J. Fish. Res. Board. Can. 32:2205-2209.

H99 Thirugnanam, M. and A.J. Forgash. 1975. Environmental impact of mosquito pesticides: Influence of temefos on the brain acetylcholinesterase of killifish. Environ. Physiol. Biochem. 5:451-459.

H100 Tooby, T.E. and F.J. Durbin. 1975. Lindane residue accumulation and elimination in rainbow trout (*Salmo gairdnerii* Richardson) and roach (*Rutilus rutilus* Linnaeus). Environ. Pollut. 8:79-89.

H101 Valentine, D.W. and M. Soule. 1973. Effect of p,p'-DDT on developmental stability of pectoral fin rays in the grunion, *Leuresthes tenuis*. Fishery Bull. (U.S. Nat'l Marine Fisheries Service) 71:921-926.

H102 Veith, G.D. 1975. Baseline concentrations of polychlorinated biphenyls and DDT in Lake Michigan fish, 1971. Pestic. Monit. J. 9:21-30.

H103 Veith, G.D., D.W. Kuehl, and J. Rosenthal. 1975. Preparative method for gas chromatographic-mass spectral analysis of trace quantities of pesticides in fish tissue. J. Assoc. Off. Anal. Chem. 58:1-4.

H104 Waggoner, J.P. and M.G. Zeeman. 1975. DDT: short term effects on osmoregulation in black surfperch (*Embiotoca jacksoni*). Bull. Environ. Contam. Toxicol. 13:297-300.

H105 Watkins, J.O. and J.D. Yarbrough. 1975. Aldrin and dieldrin uptake in insecticide-resistant and susceptible mosquitofish (*Gambusia affinis*). Bull. Environ. Contam. Toxicol. 14:731-737.

H106 Weis, J.S. and P. Weis. 1975. Retardation of fin regeneration in *Fundulus* by several insecticides. Trans. Am. Fish. Soc. 104:135-137.

H107 Weis, P. and J.S. Weis. 1974. Cardiac malformations and other effects due to insecticides in embryos of the killifish, *Fundulus heteroclitus*. Teratology 10:263-267.

H108 Weiss, C.M. 1959. Response of fish to sub-lethal exposures of organic phosphorus insecticides. Sewage Ind. Wastes. 31:580.

H109 Wojcik, D.P., W.A. Banks, W.B. Wheeler, D.P. Jouvanaz, Van Middelem, and C.S. Lofgren. 1975. Mirex residues in nontarget organisms after application of experimental baits for fire ant control, southwest Georgia -1971-72. Pestic. Monit. J. 9:124-133.

H110 Yap, H.H., K.K. Cutkomp, and R.B. Koch. 1975. *In vitro* inhibition of fish brain ATPase activity by cyclodiene insecticides and related compounds. Bull. Environ. Contam. Toxicol. 14:163-167.

H111 Young, D.R. and T.C. Heesen. 1976. Inputs of chlorinated benzenes, p. 31-37. *In* Southern Calif. Coastal Water Research Project -Annual Rept., El Segundo, Calif.

The occurrence of some chlorinated benzenes is on the same order of magnitude as PCB's in municipal wastewaters. These mitostatic poisons should be monitored more closely in the future.

H112 Young, D.R., D.J. McDermott, and T.C. Heesen. 1976. DDT in sediments and organisms around southern California outfalls. J. Water Pollut. Control. Fed. 48: 1919-1928.

H113 Zitgo, V., P.M.K. Choi, D.J. Wildish, C.F. Monoaghan, and N.A. Lister. 1974. Distribution of PCB and p,p'-DDE residues in Atlantic herring (*Clupea harengus harengus*) and yellow perch (*Perca flavescens*) in eastern Canada 1972. Pestic. Monit. J. 8:105-109.

I SEWAGE AND WASTEWATER

I1 Allen, G.H. 1976. Rearing Pacific salmon in saltwater ponds fertilized with domestic wastewater. Data Report, Humbolt State Univ.

High quality coho smolts were produced but high temperature-induced vibriosis killed chinook salmon during smolting. Supplemental feeding was necessary.

I2 Anon. 1972. The effects of waste disposal in the New York Bight. Summary Final Report. Middle Atlantic Coastal Fisheries Center. NOAA.

I3 Anon. 1972. The effects of waste disposal in the New York Bight. Section 2. Benthic studies. National Marine Fisheries Service, Highlands N.J. Sandy Hook Lab. (407296). NTIS Report AD-739 532.

I4 Arthur, J.W., R.W. Andrew, V.R. Mattson, D.T. Olson, and G.E. Glass. 1975. Comparative toxicity of sewage-effluent disinfection to freshwater aquatic life. Environmental Research Lab., Duluth, Minn. EPA/600/3-75-012.

Fish were more sensitive than invertebrates to chlorinated effluents and all species were more affected by chlorinated effluents than by nondisinfected, dechlorinated or ozonated effluents. 7-day TL_{50}'s of residual chlorine for fish were 0.08 to 0.26 mg/l and 0.21 to, 0.81 mg/l for invertebrates.

I5 Babinchak, J.A., J.T. Graidoski, S. Dudley, and M.F. Nitkowski. 1977. Distribution of faecal coliforms in bottom sediments from the New York Bight. Mar. Pollut. Bull. 8:150-153.

The area which exceeded 100 FC/ 100 ml in sediments extended 11 km north and 37 km south of the disposal site center. 82.3% of the fecal coliform group were *E. coli*.

I6 Bonde, G.J. 1967. Pollution of a marine environment. J. Water Pollut. Control Fed. 39:R45-R63.

An examination of the channel adjacent to Copenhagen produced mapping of the pollutional load; hydrography, bacterial composition of water, sediments and fish; as well as the effects on the biology and fisheries.

I7 Callahan, R.A. 1974. Apparatus and procedure for measuring sublethal toxicity of waste water discharges. NTIS Report AD-787 456.

I8 Clarke, N.A., G. Berg, P.W. Kabler, and S.L. Chang. 1964. Human enteric viruses in water: source, survival, and removability, p. 523-542. *In* W.W. Ecketelder (ed.), Advances in water pollution research, Vol. *2*, Pergamon Press, London.

The ratio of enteric virus units to coliforms in human feces is 15/million. These viruses survive longer in either unpolluted or grossly polluted waters. Activated sludge process removes 90-98% of enteric viruses in raw sewage, chemical flocculation removes 95-99% and HCLO and HIO are "highly effective in inactivating viruses in water." Infectious hepatitus is more resistant to chlorine than coliform or enteric pathogenic bacteria.

I9 Clarke, N.A. and P.W. Kabler. 1964. Human enteric viruses in sewage. Health Lab. Sci. 1:44-49.

The only known sewage-contaminated outbreak of a virus disease are due to hepatitus. The virus/coliform ratio in sewage is 1/100,000. An improved technique for virus sampling is by use of a gauze pad from which virus is eluted and concentrated. Primary sewage treatment has no effect on enteric viruses, and secondary treatment or trickling filters removes only 40% of viruses.

I10 Dunstan, W.M. and D.W. Menzel. 1971. Continuous cultures of natural populations of phytoplankton in dilute, treated sewage effluent. Limnol. Oceanog. 16:623-632.

Phytoplankton cultured in a chemostat were efficient in removing nitrogen and phosphorus from sewage and produced large quantities of plant carbon.

I11 Esvelt, L.A., W.J. Kaufman, and R.E. Selleck. 1973. Toxicity assessment of treated municipal wastewaters. J. Water. Pollut. Control Fed. 45:1558-1572.

Biological waste treatment greatly removed toxicity from primary effluents. Methylene blue active substances and NH_3 - N were found to be significant toxicants in nonchlorinated effluents. 96 hour TL_{50} of residual chlorine for golden shiners was 0.2 mg/l. Effluent toxicities are discussed in terms of toxicity units - the reciprocal of the 96 hour TL_{50} expressed as percent of sewage in the assay water.

I12 Hancock, H.M. 1974. Investigations of the influence of the effluent from a concentrated industrial complex on a large river. NTIS Report COM-74-11345/7.

I13 Greene, J.C., T.E. Maloney, W.E. Miller, and T. Shiroyama. 1975. Utilization of algal assays to assess the effects of municipal, industrial, and agricultural wastewater effluents upon phytoplankton production in the Snake River system. Water, Air, Soil Pollut. 4:415-434.

I114 Grigg, R.W. and R.S. Kiwala. 1970. Some ecological effects of discharged wastes on marine life. Calif. Fish Game 56:145-155.

"The number of macroscopic species present at five stations ranging in depths from 45 to 65 ft off the Palos Verdes Peninsula, near San Pedro, Calif., was negatively correlated to the amount of fine grain organi-laden sand present in the sediment."

I15 Koditschek, L.K. and P. Guyre. 1974. Antimicrobial-resistant coliforms in the New York Bight. Mar. Pollut. Bull. 5:71-74.

Viable fecal coliforms which are resistant to antibiotics and heavy metals exist in sediments and to a lesser extent in the overlying waters of the New York Bight. The health hazzards connected with unexplored facets of bacterial populations at sewage dump sites are discussed.

I16 Kouyoumjian, H.H. 1975. The possible use of planaria in bioassays of uric acid and sewage. Rev. Int. Oceanogr. Med. 37-38:89-95.

I17 Kumpf, H.E. 1977. Economic impact of the effects of pollution on the coastal fisheries of the Atlantic and Gulf of Mexico regions of the United States of America. FAO Fisheries Technical Paper No. 172. 79 p.

The impact of pollution on commercial fisheries is estimated at 8% of the total finfish and shell fish catch; sportfisheries losses were difficult to estimate. Fish kills caused by pollution were responsible for the major dollar losses.

I18 Limbaugh, C. 1957. Marine outfall studies by qualified diving investigators. Proc. 12th Ind. Waste Conf., Purdue Univ. Eng. Ext. Ser. 94:210-216.

Description of SCUBA gear and its use in direct observation and investigation of the benthic floral and faunal effects from outfalls.

I19 Ludwig, H.F. and P.N. Storrs. 1970. Effects of waste disposal into marine waters. A survey of studies carried out in the last ten years. Water Res. 4:709-720.

General and perhaps oversimplified discussion of results of engineering companies on effects of ocean outfalls. The need for sub-lethal effects from low concentrations of toxicants is argued.

I20 Mathur, R.P. and S.R. Verma. 1974. Studies on the toxicity of industrial wastes to *Macrobrachium dayanum*. Indian J. Environ. Health. 16:1-11.

I21 McNulty, J.K. 1970. Effects of abatement of domestic sewage pollution on the benthos, volumes of zooplankton, and the fouling organisms of Biscayne Bay, Florida. University of Miami Press, Coral Gables, Florida 107 p.

Four years after untreated sewage outfalls were removed, the elevated number of benthic macroinvertebrate species and individuals on hard bottoms decreased to normal populations. Depleted fish populations did not return to normal levels.

I22 Norris, D.P., L.E. Birke, Jr., R.T. Cockburn, and D.S. Parker. 1973. Marine waste disposal - a comprehensive environmental approach to planning. J. Water Pollut. Control. Fed. 45:52-70.

Results of an extensive study indicated that the marine environment can be adequately protected by discharging chlorinated primary effluent through outfalls with diffuser systems.

I23 O'Connor, J.S. 1975. Contaminant effects on biota of the New York Bight. Proceedings of the Gulf and Caribbean Fisheries Institute, Twenty-eighth Annual Session, October, 1975:50-63.

Documented effects of sewage disposal in New York Bight include high prevalence of diseases of fish and shellfish, degradation of benthic invertebrate communities, contamination by bacteria many of which have resistance transfer factors to antibiotics and heavy metals, and noxious concentrations of particulates, flotsam and surface slicks.

I24 Officer, C.B. and J.H. Ryther. 1977. Secondary sewage treatment versus ocean outfalls: An assessment. Science 197:1056-1060.

Simplified models were developed which enabled evaluation of both the BOD of wastewater and the secondary eutrophication resulting from release of nutrients into the marine environment. It was felt that secondary treatment of sewage was unnecessary and that more understanding of the effects of persistant trace contaminants was necessary.

I25 Pearce, J.B. 1971. Indicators of solid waste pollution. Mar. Pollut. Bull. 2:1.

Tubes constructed by the polychaete *Ampharete acutifrons* contained human artifacts and sewage sludge debris and thus were indicators of areas which were previously contaminated and the organic debris subsequently washed away by current and wave action.

I26 Pearce, J.B. 1972. The effects of solid waste disposal on benthic communities in the New York Bight. *In* M. Ruivo (ed.), Marine pollution and sea life. Fishing News (Books) Ltd., London.

A lack of normal benthic fauna exists in the 20 mi^2 sludge dumping area of N.Y. Bight. Surrounding these areas are benthic communities of low diversity and high dominance of resistant species. Crabs exposed to these sediments in the lab developed exoskeletal erosion in six weeks.

I27 Pearce, J.B. 1976. Our coastal waters: An endangered zone. Science Teacher. 43:9.

General discussion of the problems of coastal pollution in dense population areas.

I28 Pratt, S.D., S.B. Salia, A.G. Gaines, Jr., and J.E. Krout. 1973. Biological effects of ocean disposal of solid waste. NTIS Report COM-73-11292/2.

I29 Rosen, H.M. 1976. Wastewater ozonation: A process whose time has come. Civil Eng.-ASCE. 6:65-69.

I30 Swartz, R.C., J.D. Walker, W.A. Deben, and F.A. Cole. 1975. Structural analysis of stressed marine communities. EPA Water Quality Research Sym. Ore. Aug. 17-22:3-12.

I31 Topping, G. 1976. Sewage and the sea, p. 303-351. In R. Johnson (ed.), Marine pollution. Academic Press.

General oceanographic background and comprehensive discussion of sewage polluted areas of Europe and the U.S. Conclusions are that only the estuaries and smaller bodies of water have thus far been affected but that stresses are increasing.

I32 Tsai, C. 1970. Changes in fish populations and migration in relation to increased sewage pollution in Little Patuxent River, Maryland. Chesapeake Sci. 11:34-41.

Downstream of chlorinated sewage effluents the number of species and fish abundance decreased. Further down in areas of organic richness the number of species remained the same but the composition of the fish communities varied.

I33 Tsai, C. 1973. Water quality and fish life below sewage outfalls Trans. Am. Fish. Soc. 102:281-292.

Studies of 149 secondary treated sewage outfalls in Virginia, Maryland and Pennsylvania showed that chlorine and increased turbidity reduced fish species diversity downstream of the outfall.

I34 Tsai, C. 1975. Effects of sewage treatment plant effluents on fish: A review of the literature. CRC Publication No. 36. 220 p.

Excellent review pointing out the great lack of knowledge of toxicants in the marine system. The effects of chlorine and the chemical species formed from it in seawater, and the formation of unionized ammonia in seawater are little known. Synthetic detergents may be more toxic and H_2S occur in greater concentration in the marine environment than in freshwater.

I35 Valiela, I., M.D. Banus, and J.M. Teal. 1974. Response of salt marsh bivalves to enrichment with metal-containing sewage sludge and retention of lead, zinc, and cadmium by marsh sediments. Environ. Pollut. 7:149-157.

I36 Ward, R.W., R.D. Giffin, G.M. DeGraeve, and R.A. Stone. 1976. Disinfection efficiency and residual toxicity of several wastewater disinfectants. Vol. 1. Grandville, Michigan, EPA, Cincinnati, Ohio. EPA-600/2-76-156.

Exploration of toxicity problems from wastewater chlorination and the alternatives of ozonation, bromine chloride treatment and dechlorination. Dechlorinated, ozonated and nondisinfected effluents proved nontoxic in acute and life cycle bioassays while bromine chloride was generally toxic in concentrated effluents. Chlorine was the most toxic of all.

I37 Widmer, W.J., F.R. Trainor, J.D. Buck, A.W.H. Damman, and W.R. Whitworth. 1975. Biotic changes in the Willimantic/Shetucket River associated with improvements in sewage treatment. Office of Water Research and Technology, Wash., D.C. NTIS Report PB-249 817/8ST.

I38 Wilber, C.G. 1971. The biological aspects of water pollution. 2nd ed. Charles C. Thomas, Springfield.

Basic background information on the associated problems of water pollution covering domestic and industrial sewage, metals, pesticides, oil, radionuclides and pulp mills

I39 Williamson, K.J. 1976. A bioassay to assess wastewater toxicity to aerobic biological treatment. Office of Water Research and Technology, Washington, D.C. OWRT-A-036-ORE. NTIS Report PB-261 394/1ST.

I40 Young, R.H.F., G.L. Dugan, L. Lau, and H. Yamauci. 1975. Eutrophication and fish toxicity potentials in a multiple-use subtropical reservoir. Office of Water Research and Technology, Wash., D.C. NTIS Report PB-263 089/5ST.

J SULFUR COMPOUNDS

J1 Bella, D.A. 1974. Benthal Sulfide release in aquatic systems. NTIS Report PB-237 074.

J2 Smith, L.L., Jr., D.M. Oseid, I.R. Adelman, and S.J. Broderius. 1976. Effect of hydrogen sulfide on fish and invertebrates. Part I - Acute and chronic toxicity studies. EPA Report 600/3-76-062a.

Comprehensive study which determined that 0.002 mg/l H_2S would protect all freshwater fish and bottom invertebrates. Toxicity varied with species, source of fish and temperature of bioassays.

J3 Smith, L.L., D.M. Oseid, and L.E. Olson. 1976. Acute and chronic toxicity of hydrogen sulfide to the fathead minnow Pimephales promelas. Environ. Sci. Technol. 10:565-568.

The 96 hr LC_{50} of lab-cultured fathead jinnow juveniles at 20°C was 0.0243 mg/l. Wild stock were more resistant at lower temperatures and less resistant at higher temperatures.

K SUSPENDED SOLIDS

K1 Sherk, J.A., J.M. O'Connor, D.A. Neumann, R.D. Prince, and K.V. Wood. 1974. Effects of suspended and deposited sediments on estuarine organisms, phase II. NTIS Report AD-A011 372.

L TOXICANTS miscellaneous and general

L1 Anon. 1976. Quality criteria for water. EPA-440/9-76-023.

Deals with effects of most significant water constituents and pollutants. There is an introduction, rationale, references and recommended criterion for each constituent.

L2 Cardwell, R.D., D.G. Foreman, T.R. Payne, and D.J. Wilbur. 1976. Acute toxicity of selected toxicants to six species of fish. EPA/600/3-76/008. NTIS Report PB-252 488/2ST.

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APPENDIX C

OPERATIONAL PROCEDURES MANUAL FOR STABEL EXPERIMENTS

Dr. Lanny R. Udey

The following material was provided by Dr. Lanny R. Udey, Department of Microbiology, School of Medicine, University of Miami. Sections 12 and 13 have been re-typed to improve legibility, and headings for Section 9 to 14 have been added.

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NOTICE

All personnel and investigators using these facilities will be required to receive inoculations for typhoid, polio, and tetanus. In addition, recommendations from the federal report on Occupational Health and Safeth Standards for General Industry will be followed.

1. SCHEDULING OF EXPERIMENTS

1.1 The lab manager must schedule the use of all fish tanks. A list will be kept on the bulletin board of all planned experiments so that the manager can indicate when an experiment may be initiated.

1.2 It may be necessary to conduct regular meetings (monthly) with personnel utilizing the lab facilities in order to efficiently schedule the use of the tanks.

1.3 The lab manager must be notified as early as possible of an intent to use the lab facilities. The following information should be provided:

a. Total number of fish and species.
b. Number of tanks necessary (stock and experimental).
c. Projected length of the experiment.

1.4 Tanks not in use or not scheduled for use should be marked so and their existence made known by the lab manager to those experimenters planning to use the facilities.

1.5 When an experiment is to be conducted with a particular sewage treatment and dilution, tanks previously used with this same treatment and dilution will be rescheduled for the planned experiment.

2. PROCUREMENT OF EXPERIMENTAL ANIMALS

(do not overestimate)

2.1 Experimental animals will be collected according to the methods outlined in Appendix I: Fish Collection and Maintenance.

2.2 For experimental animals obtained from a mariculture facility, a form will be provided that must include the following information:

a. Person needing the fish.
b. Number of fish.
c. Species of fish.
d. When the fish are needed.
e. Hatchery from which the fish will be obtained.
f. Short explanation of what the fish are going to be used for.

2.3 The form must be signed by Dr. Udey and then sent to the agency from which the fish are requested at least one week prior to when the fish are needed. In some cases, the request will be made by telephone but this must be followed by a written request.

2.4 When permission is obtained, the lab manager or person obtaining the fish should contact the hatchery manager as early as possible concerning the intended date of transfer.

2.5 Before the actual transfer the person requesting the fish and the lab manager should decide on the method of transfer, the acclimation technique, the stock tank space necessary, and the length of time that the fish are held in the stock tanks before being used in an experiment. Occasionally, two people are necessary to transfer the fish properly; hence the person requesting the fish should assist the lab manager.

2.6 Upon arrival of the fish to the lab, the lab manager should record the following data on a printed form and this record kept on file at the lab.

a. Date transferred.
b. Intended experimental use and experimenter.
c. Source.
d. Number.
e. Species.
f. Age and weight.
g. Stock tank numbers.
h. Daily mortalities(recording the number of each stock tank individually). This record will be kept on a special mortality sheet for the stock tanks.
i. Disposition of fish to experiments, including date, experimenter, experiment number and number of fish.

2.7 Fish introduced into the stock tanks must be quarantined and examined for disease. The procedure is outlined in section 11.4 of the NTIS report on Standard Practice for Acute Toxicity Tests to Fish, Macro-invertebrates, and Amphibians (see Appendix II).

2.8 Prophylactic treatment of marine fish is to be avoided if possible as disruption of the normal flora can increase susceptibility to infection. However if losses are greater than 5% per day in the first three days and the problem is not viral, treatment can proceed according to Appendix III.

2.9 Animals introduced into the stock tanks must be held two weeks in flowing dilution water before they are used in an experiment.

2.10 A natural photoperiod will be maintained by the design of the facility. Electric lights will not be turned on after dark unless necessary.

3. DISINFECTION OF THE FISH TANKS AND OTHER POTENTIALLY CONTAMINATED AREAS

3.1 All surfaces which are exposed to fish pathogens and are potential sites of contamination should be disinfected.

3.2 Tanks used in disease experiments will be disinfected by the following steps:

a. The tanks should always be disinfected immediately after they are used.

b. Rubber gloves should be worn while disinfecting the tanks.

c. The tank is rinsed out.

d. The tank is scrubbed with chlorinated detergent (HC-10 Klenzade, two oz./gal. water) using a nylon brush, drained and rinsed.

e. Next, the interior of the tank is filled with Iodophor solution (25ppm iodine or 0.55 1/100 gal. tank of Klenzade ID-11) and allowed to stand for two hours.

f. The tank is then given a good rinse with water to remove any Iodophor residue and the normal water flow resumed for at least 3 hours prior to introduction of fish.

g. Subsequent to being soaked and scrubbed in detergent

3. DISINFECTION OF THE FISH TANKS AND OTHER POTENTIALLY CONTAMINATED AREAS (cont'd)

the lids and other parts are sponged with the Iodophor solution (25 ppm iodine) and rinsed as above.

h. The tank is made ready for the next experiment. If a tank has been disinfected but not used within one week, it should be filled with Iodophor solution (25 ppm iodine) for one hour the day before the experiment is initiated, then the normal flow of water resumed.

3.3 The stock tanks should be cleaned whenever the fish are removed prior to introducing new fish. The same procedure of disinfection will be used.

3.4 When an experiment is in progress, usually twice a week the tanks will need to be cleaned of debris. Possibly the best time to clean tanks is when the dead fish are removed. Rubber gloves must be worn during this procedure. Control tanks should be cleaned first followed by the experimental tanks in an order designated by potential disease virulence. In general, tanks containing the two chlorinated secondary treatments (from lowest to highest sewage concentration) are done first. Following this, the chlorinated primary treated sewage tanks (from lowest to highest sewage concentration) are cleaned. The non-chlorinated treatments are done in the same manner; secondary first, then primary. An exact order of numbered tanks based on the current experimental design will be posted on the bulletin board and changed as necessary.

A container of disinfectant on wheels kept in the wet lab area should be brought next to the tank. First the outer pipe (screen) is removed from the tank, held over the disinfectant and brushed and sprayed clean with fresh water. The same procedure is followed with the standpipe. Then a special Klenzade nylon sponge brush (three feet long) will be used to loosen the scum from the bottom of the tank. A large supply of these disinfected brushes will be kept in the lab, one brush per experimental treatment. When a brush has been used it is immediately immersed in the container of disinfectant and left there for one-half hour. Then this brush should be rinsed thoroughly with water. With the standpipe raised to allow water to leave the tank, the brush is used to swirl the water and debris in the tank until all debris has been removed from the tank.

3.5 The wet lab and stock tank decks will be sanitized twice weekly with a chlorinated detergent (Klenz-Klor Z-3 Klenzade). The detergent is sprinkled on the deck, wetted down, then brushed off into the trenches. This procedure will be followed on days that disease experiments are initiated and when disease agents are known to have been spilled on the deck.

3.6 Any pails, lab carts, etc. used in the contaminated area must be disinfected with Iodophor solution subsequent to use. A plastic container filled with an Iodophor solution (25 ppm iodine) will be kept in the wet lab area at all times. The person using this equipment must be sure it is disinfected.

3.7 The freezers and refrigerators should be cleaned once a month and defrosted as required.

3.8 Traffic in the stock tank and wet lab areas should be held to a minimum.

3.9 No one will be allowed to work in the stock tank area unless they are wearing rubber gloves rinsed in Iodophor solution.

3.10 No stock tanks should be opened unless necessary.

3.11 Both large and small nylon nets will be used in the stock tank and wet lab areas. The large nets used in the stock tank area should be marked "Stock Tank Only" and stored in the equipment storage room along with a supply of small nets. A tray of Iodophor solution (25 ppm iodine) will be kept in each area where nets can be immersed (30 minutes minimum). These nets should be thoroughly rinsed in fresh water prior to use. A supply of disinfected small nets will be kept in a drawer in in the dry lab for use in the wet and dry lab areas.

3.12 When transporting fish from the stock tank area to the disease experimental area, specially marked plastic buckets (Stock Tank Transfer Only) should be used. Subsequent to use, the buckets should be disinfected in an Iodophor solution (25 ppm iodine) for one-half hour and stored in the equipment storage room. Other buckets and containers shall be marked "Wet Lab Only" and never used in the stock tank area. These containers should be immersed one-half hour in the large plastic container of disinfectant held in the wet lab.

4. RECORD OF EXPERIMENTS

4.1 A record of all experiments will be kept at the lab. When an experiment is initiated, the person conducting the experiment must fill out an experiment description sheet (printed form) and place it on a clip board which is kept in a designated area of the wet lab. The lab manager will record daily mortalities on this sheet; and when an experiment is terminated, the sheet will be placed in a file at the lab. From this sheet the person running the experiment can either copy the data in his lab notebook or make xerox copies. The original form should be returned to the lab file.

4.2 The experiment description sheet will contain the following information:

a. Experimenter.
b. Experiment number (includes experimenter's initials)
c. Title of experiment.
d. Date initiated and terminated.
e. Control and experimental tank numbers with their respective fish diets.
f. Fish data: species, number per tank, weight (size), source, and date brought to fish lab.
g. Sewage treatment and dilution, chlorinated or

non-chlorinated.

h. Daily mortality recorded by tank number. (Separate mortality sheet).

4.3 The data card for each tank should be filled out on the day that the experiment is initiated and placed in the holder provided for each tank.

The person running the experiment should fill in the following information:

a. Tank number.

b. Experimenter.

c. Experiment number and title, sewage treatment and dilution, chlorinated or non-chlorinated.

d. Date initiated.

e. Number of fish and species.

f. Type of ration.

g. Control or experimental group.

5. FEEDING OF FISH

5.1 Before stock fish are brought to the lab, the person needing the fish should determine what feed (size) the fish are to be fed. Then he should confer with the lab manager to be sure the right feed is available at the lab.

5.2 The lab manager will be responsible for ordering the common types of feed.

5.3 Generally, all fish will be fed "ad lib" twice a day and once each day during the weekind. Feeding "ad lib" in this case will be defined as adding feed carefully and in small amounts until the feed reaches the bottom of the tank without being taken. Certain experiments will require modifications of this procedure. A person having the weekend feeding duty should be thoroughly familiar with the feeding procedures.

5.4 The daily feeding procedure will be as follows:

a. The stock tank fish will be fed first (gloves must be worn). One container of each size fish feed will be kept in the top section of the freezer. Each of these containers will be labled "Stock Tank Feed" and include the ration type and the numbers of the tanks receiving this ration.

b. For the wet lab tanks, one container of control feed will be used for all the controls (unless different rations are required for different control groups). All control tanks should be fed first, followed by the experimental tanks in the order posted.

When feeding fish in the wet lab area, gloves will be worn, and a spatula or spoon will be kept with each container of feed and this used to dispense feed into the tanks. Modifications of this procedure will be necessary when weighed amounts of feed are held in plastic bags.

5.5 Extra feed will be stored in the stock tank feed section of the freezer.

5.6 Plastic bags containing weighed portions of feed must be individually marked with the proper experiment number and tank number.

5.7 If a group of stock tanks are suffering losses thought to be of disease origin, a separate container of feed should be kept for these fish in the disease feed section of the freezer until the fish are treated and recover or are eliminated.

6. HANDLING OF DEAD ANIMALS

6.1 Dead animals will be picked up each morning after the fish are fed. The stock tanks should be picked first, then the control tank fish and so on according to the posted order. Dead animals will be picked only in the morning unless special arrangements are made with the lab manager to do otherwise. Fish near death will also be picked at this time.

6.2 Dead animals from each individual tank will be placed in a plastic bag with a tag marked with the following data:

a. Experiment number.
b. Number of tank.
c. Number of animals per bag.
d. Date.
e. Experimenter's initials.

6.3 The dead animals in each tank of each experiment will be collected and recorded on a mortality data sheet on the clip board. The plastic bags will be refrigerated immediately and examined according to Appendix IV: Autopsy Procedure. A separate container will be kept for each experiment. The containers should be marked with the experiment number, the tank numbers, and the person conducting the experiment. Fish that die as a result of lab procedures or those that jump out of a tank should be recorded as KLO on the mortality sheet and stored in a separate plastic bag.

6.4 When picking dead animals, rubber gloves should be worn and rinsed in Iodophor when going from one experiment to the next. Special nets will be provided. One net will be used for each tank and then all the nets disinfected (as described in Disinfection of Fish Tanks etc., section 3) subsequent to use. A metal tray containing Iodophor solution (25 ppm iodine) placed on a cart should be brought next to the tank so the net can be immediately placed in the disinfectant subsequent to use. A large number of disinfected nets will be kept in a specially marked area of the lab.

6.5 Dead animals in the stock tanks will be picked once a day before any of the experimental tanks. The disinfected dead animal nets will be used, one for each stock tank. A daily mortality record will be kept for each tank on a sheet held on a clip board. Used sheets will be stored in the lab file. If the daily mortality in any one of the stock tanks exceeds 0.1% of the total number of fish the lab manager should determine disposition of the fish. These fish should be autopsied immediately and a decision made as to whether the fish should be treated or sacrificed. In the case of viral infections, the fish should be disposed of immediately to prevent spread of diseases in the stock tank area.

6.6 After examination, all dead animals will be placed in plastic bags and transported to the Medical School for incineration.

7. WEEKEND PROCEDURES

7.1 Anyone with weekend duty must be thoroughly familiar with the daily schedule of procedures involved in caring for fish prior to when the weekend comes.

7.2 A weekend duty list will be kept and all who use the lab facilities will be required to take care of the fish during their particular weekend. This is to be posted several weeks in advance.

7.3 The procedure to follow during the weekend is as follows:

a. Fish will be fed and dead animals picked only once a day on Saturday and Sunday.

b. First the stock tank fish should be fed (wearing gloves).

c. Then the dead animals in the stock tanks are picked and recorded.

d. Next, all control fish in the wet lab area are fed, and finally the fish in the experimental tanks according to the posted order. Feeding technique is described in the Feeding of Fish, Section 5.

e. The dead animals are picked out in the same order.

7.4 Anyone conducting large experiments or complicated feeding schedules involving many different rations, or weighing out quantities of feed should take care of their

7. WEEKEND PROCEDURES (cont'd)

own experiment during the weekend or make sure whoever has weedend duty is thoroughly familiar with the procedure. Also, if fish have to be fed more than once a day, it will have to be done by the experimenter.

8. GENERAL COMMENTS

8.1 Anyone using the lab facilities must clean up after themselves and return all instruments and tools to their proper places!!

8.2 All laboratory work where fish are not involved should by be done in the dry lab.

8.3 Fish which are no longer needed must be sacrificed.

8.4 Whenever the supply of stock items becomes low, contact the lab manager.

8.5 Everyone running experiments in the lab should help the lab manager as much as possible in taking care of their own experiment. This includes cleaning tanks, picking up fish, feeding fish and picking dead animals. In some cases this will be absolutely necessary because of the time involved in taking care of each experiment.

9. FISH COLLECTION AND MAINTENANCE

INTRODUCTION

During the course of this study it will be necessary to collect, transport and feed several hundred live, healthy specimens of the genera *Cyprinodon*, *Fundulus*, *Menidia*, *Mugil*, *Lagodon* and *Eucinostomus* per week for controlled exposure to dilutions of secondary sewage effluent. Before this can be accomplished, the following problems must first be solved.

1. Where can the six designated species be caught in large numbers and in good health?
2. How can large numbers be collected and transported while minimizing physical injury and physiological shock?
3. When are these species most abundant and how should their collection and experimental use be scheduled?
4. What are some other significant species that can be expected to be collected in large numbers, along with the six designated species, and at what time of year are they most abundant?
5. What are the natural foods of all the experimental species and what sterile, commercial diets are best suited, both nutritionally and economically, for the long term maintenance of these species?

METHODS

Collection

A 100' x 6' x 3/8" stretch mesh bag seine, fished in grassbeds in a shore to shore semicircle, will be used to collect the large numbers of fish necessary for this study. This method should catch all six designated species while minimizing physical injury and physiological shock. Alternative capture methods, listed here in decreasing desirability, include otter trawl, trapsand hook and line. A beach seine will catch fish from all levels of the water column while not exposing them to the punctures and abrasions that occur with hooks, traps or trawls. The only possible foreseen exception to the use of a beach seine, may occur in attempts to catch large numbers of _Mugil curema_ in which case a cast net may be required. Other species, whose adult forms occur in the vicinity of ocean outfalls, will be used to supplement the data generated from this study.

In order to insure the good health and necessary controls for this study, all fish will be collected away from known point sources of pollution. The proposed area for the majority of specimen collection is Card Sound in South Biscayne Bay. This area is removed from dense urban populations and the agricultural drainage canals about eight kilometers to the north. On-site sampling for bacteria and other potential pollutants will be conducted regularly.

Transportation

Transportation of fish from the collection site to the laboratory holding tanks will be accomplished by on-site segregation of species into 24 gallon, thermally insultated coolers. Each cooler will hold only one species and will be independently aerated. Fish will always remain suspended in water in order to minimize injuries resulting from net beating, such as scale loss and eye damage as well as reducing physiological shock. The coolers will be used to transport fish from shore to the boat and from the boat to the truck waiting at Turkey Point. Upon arrival of the truck at Virginia Key, and after gradual acclimation, the fish will be poured into their appropriate holding tank. An 80 character field code of external disease symptoms will be used to eliminate specimens injured in collection and not considered suitable for experimental use.

Seasonality

Due to the natural cycles of seasonal abundancy and scarcity for the six designated species, and acting on the knowledge that these cycles vary according to geographic locale, a literature search was conducted to determine optimal collection and experimental periods for the designated species. Among the studies referred to, Low (1973),Brook (1975) and Roessler (1967) were considered most relevant due to their quantitative data on seasonal abundancy and combined geographic bracketing of the collection area. The table of species monthly collection preferences (Table C-1) is based on the relative seasonal abundancy of both the six designated species and possible supplemental species as reported in the literature cited.

The scheduling of experimental species will be subject to environmental influences that make precise predictions of some species availability difficult. Brook (1975) reported reduced fish and crustacean populations in Card Sound from November through March. The fish population did not expand until the seasonal increase in benthos started in March. Species whose collection in sufficient numbers may require more refined collection methods or travel to the Gulf Coast of Florida will be determined during the first months of collection in Card Sound.

Some species of juvenile grassbed species whose adult forms can be found at ocean outfalls include White Grunt (Haemulon plumieri), Gray Snapper (Lutjanus griseus), Red Grouper (Epinephelus morio) and Gag Grouper (Mycteroperca microlepis). Pompano (Trachinotus carolinus) can be collected by seining along shallow, sandy beaches on the Atlantic Coast from May to October. Attempts will be made to collect sufficient numbers of juvenile Dolphin (Coryphaena hippurus) from under offshore

TABLE C-1

	DESIGNATED SPECIES	SUPPLEMENTAL SPECIES
JAN.	Adult *Lagodon rhomboides*	Adult *Haemulon plumieri*
FEB.	*Penaeus duorarum*	*Anchoa mitchilli*
MARCH	*Mugil curema*	*Trachinotus carolinus*
APRIL	*Mugil curema*	*Coryphaena hippurus*
MAY	*Mugil curema* *Lagodon rhomboides*	*Coryphaena hippurus*
JUNE	*Lagodon rhomboides*	*Harengula pensacolae* *Haemulon plumieri*
JULY	*Eucinostomus gula*	*Lutjanus griseus* *Haemulon plumieri*
AUGUST	*Eucinostomus gula*	*Lutjanus griseus* *Haemulon plumieri*
SEPTEMBER	*Eucinostomus gula* *Fundulus grandis*	
OCTOBER	*Fundulus grandis* *Cyprinodon variegatus*	
NOVEMBER	*Cyprinodon variegatus* *Atherinomorus stipes*	
DECEMBER	Adult *Lagodon rhomboides*	

This table is based on relative seasonal abundancies and the projected effort that will be required to catch sufficient numbers of the species listed. All designated genera (*Atherinomorus stipes* replacing *Menidia*) will be collected.

windrows of *Sargassum* weed by means of an adjustable, shielded plankton net. Other supplemental species of sport and commercial importance, which cannot be caught in sufficient numbers or are not native to South Florida may possibly be obtained from the State of Florida Department of Natural Resources Marine Research Laboratory in St. Petersburg, Florida. These species include Snook (*Centropomus undecimalis*) and possibly Striped Bass (*Morone saxatilis*). These species will be transported back to the Virginia Key experimental site by tank truck. Ideally, experiments on those species acquired from laboratory spawnings should be run in the Winter when low abundancies and poor field conditions will decrease catches in Card Sound and the surrounding waters.

Feeding

Natural dietary preferences, for each species, were referenced in the literature with concentrated efforts being directed toward the calculation of the relative and total amounts of food required for normal body maintenance and growth. Swanson found that a mean consumption of 4.8% of the body weight of the Gray Snapper (*Lutjanus griseus*) was required daily between 25°C and 35°C. Assuming 5%/day for 1,500 fish, each 80 mm long and 100 gms in weight, a total of 53 kg of food/week would be required. Over a one-year period a total of approximately 2,750 kg of food will be required.

Before firm agreements are made with commercial food sources, bacterial, viral, heavy metal and pesticide analyses will be conducted. A high priority is being given to eliminate any potential source of disease that may be present in food entering the experimental and holding tanks. Fish will, tentatively, be fed a combination of Purina Marine Chow and Gamma Frozen Diets. The Purina Marine Chow is available in different pellet sizes with both 20% and 25% protein content and is presently undergoing testing by Purina Foods at Crystal River on the West Coast of Florida. It is not unrealistic to assume that a mutually advantageous working relationship will be established with Purina and modifications of pellet composition may be possible to suit the nutritional needs of *Mugil*, *Eucinostomus*, or other species should it become necessary. The less expensive Purina pellets will be supplemented with the frozen, natural foods from Gamma Foods with 50% protein content. Their extensive line of sterile, gamma irradiated, foods will provide variety and stimulate newly collected fish to begin feeding in captivity. All fish species will receive a vitamin and trace element supplement to supply complete nutrition and insure that decreased health does not result from inadequate diet. Formulas used successfully in aquaculture projects will be used when applicable to reduce feeding costs. Rations for all species will be labeled and pre-packaged to maintain a consistent quantitative and qualitative diet that will not be subject to variation.

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11. ACCLIMATION OF TEST ORGANISMS

From The NTIS Report On
Standard Practice for Acute Toxicity Tests
To Fish, Macro-Invertebrates, and Amphibians

11.4 Care and handling. Organisms must be cared for and handled properly (18) to avoid unnecessary stress.

11.4.1 To avoid unnecessary stress, organisms should not be subjected to rapid changes in temperature or water quality. In general, aquatic organisms should not be subjected to more than a 3°C change in water temperature in any 12-hour period. Between use for different groups of test organisms, holding and acclimation tanks should be sterilized with an iodophor or with 200 mg of hypochlorite/litre for 1 hour, brushed well once during the hour, and then rinsed well. Although iodophors are not acutely toxic to most aquatic animals, hypochlorite is. Hypochlorite can be removed by flushing or by reacting it with sodium thiosulfate or sodium sulfite. Organisms first brought into the facility should be quarantined for at least 7 days and until they appear to be disease-free. To maintain organisms in good condition during holding and acclimation, crowding should by avoided and the dissolved oxygen concentration must be maintained between 60% and 100% saturation (16); gentle aeration may be used if necessary.

11.4.2 Organisms should be fed at least once a day and tanks scraped or brushed at least twice a week.

11.4.3 Organisms should be handled as little as possible. When handling is necessary, it should be done as gently, carefully, and quickly as possible, so that the organisms are not unnecessarily stressed. Organisms that touch dry surfaces or are dropped or injured during handling must be discarded. Dipnets are best for handling larger organisms. Such nets are commercially available, or can be made from small mesh nylon netting, nylon or silk bolting cloth, plankton netting, or similar material. Nets coated with urethane resin are best for handling catfish. Smooth glass tubes with rubber bulbs should be used for transferring smaller organisms such as daphnids or midge larvae. Equipment used to handle aquatic organisms should be sterilized between uses by autoclaving or by treatment with an iodophor, 200 mg of hypochlorite/litre, or 30% formalin plus 1% benzalkonium chloride for one hour. Hands should be washed or sterilized before handling or feeding test organisms.

12. A COMPREHENSIVE LIST OF THE MOST IMPORTANT DISEASES OF FISHES -- DRUGS AND CHEMICALS USED FOR CONTROL OF DISEASES -- AND RECENT ENGLISH REFERENCES ON DISEASES OF FISHES

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The following tables contain an alphabetical list of the most important diseases of fishes caused by viruses, bacteria, plant and animal parasites -- with most frequently used methods of control.

Tables listing drugs and chemicals (alphabetized) include both generic names and the most common trade names. While the same drug or chemical may be used in products with different trade names, the different trade names usually represent different formulations of the chemical. Often these formulations contain drugs in diluted forms and are most convenient in application. Concentrations recommended in the right hand column show the quantity of an undiluted drug. Therefore, when using any drug, or chemical, available in specific formulations, users are advised to follow the instructions available with the drugs, and to take under consideration the concentration of the drugs present in trade-name formulations.

TABLE C-2 LIST OF THE MOST IMPORTANT VIRUS DISEASES OF FISHES

Revised by K.E. Wolf, May 1975

Disease	Causative Agent	Control
	Diseases Caused by Viruses	
Channel catfish virus disease	Channel catfish herpesvirus	Avoidance of infection. Disinfection of eggs with iodophors.
Epitheliocystis	Bedsonia-like agent	Avoidance
Infectious hematopoietic necrosis. Salmonids	Infectious hematopoietic necrosis herpesvirus	Avoidance. In some cases water temperature above 15 C.
Infectious pancreatic necrosis. Salmonids	Infectious pancreatic necrosis virus	Avoidance. Egg disinfection with iodophors. Susceptibility decreases with age.
Lymphocystis disease. Effects wide range of freshwater and marine fishes mainly during the warm season of the year.	Lymphocystis virus	Avoidance
"Red disease." Erythema of pike in Europe	Rhabdovirus species	Avoidance
Salmonid herpesvirus disease	Herpesvirus salmonis	Avoidance
Spring viremia of carp. So far reported only in Europe	Rhabdovirus carpio	Avoidance
Viral hemorrhagic septicemia. Mainly rainbow trout in Europe	Egtved virus	Avoidance

TABLE C-2 (continued)

Disease	Causative Agent	Control
Diseases of Doubtful Viral Etiology		
Carp erythrodermatitis in Europe	A virus, myxobacteria, or a mixed infection	Avoidance
Eel papilloma (Cauliflower disease stomatopapilloma)	Virus isolated but its etiologic role unconfirmed	Avoidance
Fish pox	Possibly a herpesvirus	Avoidance
Ulcerative dermal necrosis (U.D.N.) salmonids in Europe. A disease of questionable nature and pathology	Etiology unknown	Infectious nature unconfirmed

TABLE C-3 DISEASES CAUSED BY BACTERIA

Acinetobacter as cuase of losses in channel catfish eggs	*Acinetobacter* sp.	Iodine in form of iodophors. 10 min bath in iodophor at 100 ppm iodine
Bacterial gill disease Mainly in hatchery salmonids	Unidentified myxobacteria when fish kept in crowded conditions	Avoidance of crowding Furanace added to water water at 1 ppm for 1 hr Roccal, Hyamine, and other quaternary ammonia disinfectants Diquat
Bacterial hemorrhagic speticemia Worldwide occurrence Also red leg disease of frogs	*Aeromonas liquefaciens* (Synonyms: *A. punctata*; *A. hydrophila*)	Avoidance of infection Oxytetracycline or chloramphenicol orally or intraperitoneally Furanace Egg disinfection with iodophors
Coldwater disease salmonids	*Cytophaga psychrophila*	Oxytetracycline orally Sulfamethazine or sulfisoxasole orally Quaternary ammonia or or Diquat externally Chlortetracycline added to water Furanace added to water
Columnaris disease worldwide occurrence (Cottonmouth disease)	*Chondrococcus columnaris*	Avoid crowding, rough rough handling, and temperature above optimum for species Treatment as coldwater disease Oral immunization very promising
Emphysematous putrefactive disease of channel catfish	*Edwardsiella tarda*	Oxytetracycline orally Avoid crowding
Fin rot	Multiple causes	Avoid crowding and water pollution Furanace bath

TABLE C-3 (continued)

Fish furunculosis Worldwide except Australia and New Zealand. Chiefly disease of salmonids	*Aeromonas salmonicida*	Sulfamerazine Oxytetracycline Furoxone Nifurprazine in water or with food Chloramphenicol Disinfection of eggs with iodophors Sulfisoxasole in brown trout Oral immunization (only under experimental conditions)
Flavobacterium disease of channel catfish eggs	Unidentified species of *Flavobacterium*	Iodine in form of Betadine. Bath in 10 to 100 ppm iodine for 10 min.
Kidney Disease (Dee disease) In salmonids in North America and Europe	*Corynebacterium* inadequately described (possibly a *Listeria*)	Avoid exposure Prophylaxis with sulfamethazine
Mycobacteriosis Univeral -- Common in aquaria	*Mycobacterium piscium* *M. fortuitum* and related	Avoid infection Kanamycin with food
Nocardiosis (similar to mycobacteriosis) Universal distribution but not very common disease	*Nocardia asteroides*	Avoid infection
Pasteurellosis mostly marine and estuarine fishes -- worldwide, not common	*Pasteurella piscicida*	Avoid infection
Pseudomonas disease (very similar to Bacterial hemorrhagic septicemia)	*Pseudomonas fluorescens* capsulated form very virulent	As in Bacterial hemorrhagic septicemia
Redmouth disease (rainbow trout in the United States	R.M. enteric bacterium	Sulfamerazine Oxytetracycline Chloramphenicol Oral immunization

TABLE C-3 (continued)

Streptococcus infection in pondfish culture	*Streptococcus* sp.	Avoidance Avoid heavy crowding at high temperature
Ulcer disease chiefly brook trout the United States	*Hemophilus piscium*	Oxytetracycline, and chloramphenicol, orally
Vibriosis Worldwide, mostly in marine and estuarine environment also aquarium fishes	*Vibrio anguillarum*	Oxtetracycline, nitrofurazone and sulfamerazine, orally Furanace in bath externally Nifurprazine as a bath Sulfamethazine Oral immuzination Avoid infection
Vibriosis of humans from fish. Common in Japan.	*Vibrio parahaemolyticus*	Avoid raw or poorly cooked seafood

TABLE C-4 DISEASES CAUSED BY PARASITES

Actually, viruses and bacteria are also parasites. It is customary, however, to consider other parasites separately.

Numbers of parasites of fishes are very large and only the most common types are listed here. For specific information, readers are referred to the following detailed sources: Hoffman, 1967; Sindermann, 1970; Amlacher, 1970; Godlstein, 1971; Hoffman and Meyer, 1974.

	Fungi	
Branchiomycosis	*Branchiomyces* *B. sanguinis* *B. demigrams*	1. Avoidance 2. Disinfection of ponds with quicklime, calcium cyanamide, or copper sulfate
Ichthyophonus disease	*Ichthyophonus hoferi* (*Ichthyosporidium hoferi*)	1. Avoidance 2. Do not use contaminated fish as fish food
Saprolegniosis (as used in its broad meaning)	Species of *Saprolegnia*, *Achyla* and other	1. Filtration of water 2. Disinfection of water 3. Malachite green 4. Formalin
	Algae - Dinoflagellates	
Oodiniosis	*Oodinium* *O. ocellatum* *O. pillularis*	1. Cleanliness of tanks 2. Copper sulfate 3. Malachite green 4. Malchite green with formaline 5. Acriflavine 6. Increase temperature to 30 C
Diseases caused by green algae are not common		

TABLE C-4 (continued)

External Protozoans

External protozoans are very common parasites of fishes causing frequent losses. The most common are:

	Costia Chilodonella Trichodina Ambiphrya Epistylis Trichophrya	1. Avoidance 2. Formalin 3. Copper sulfate 4. Malachite green 5. Methylene blue 6. Formaline with Malachite green

Intradermal Protozoans

Ichthyophthiriasis ("Ich")	Ichthyophthirius	1. Avoidance 2. Killing of free-swimming forms with: a. Formalin b. Malachite green c. Formaline with Malachite green d. Copper sulfate e. Temperature as high as fish will tolerate

Intestinual Protozoans

Inhabiting of lumen of the intestines.

Hexamitiasis	Hexamita	1. Enheptin 2. Cyzine 3. Carbarsone oxide 4. Dimetridazole

Systemic Protozoan Parasites

Systemic protozoan parasites are very common in nature. In cultured populations of fishes control of infection by systemic protozoan parasites is feasible by observance of strict sanitary measures. Treatment of infected fishes is as yet not practical. The most common are:

	Cryptobia, Trypanosoma, Babesiosoma, Myxosporidae, Ceratomyxa in salmon. Myxosoma, Myxobolus, Henneguya, Thelohannelus, Hofferulus, Nosema, Glugea, Plistophora, Thelohania	These parasites are seldom, if ever, transmitted directly from fish to fish. They can be transmitted by feeding infected fishes, by other parasites, as leeches crustaceans,

TABLE C-4 (continued)

		or require aging of spores under natural conditions. The only practical control is by avoidance. Chemotherapy is not sufficiently advanced but promising. Disinfection of bond bottoms with quicklime or calcium cyanamide

External Trematode Worms or Monogenetic Flukes

are common and bothersome parasites. There are hundreds of species of which the most common belong to the following genera: Gyrodactylus Dactlylogyrus Cleidodiscus Benedenia They are easily transmitted directly from fish to fish and by water.		The best control is avoidance. They can be controlled with Formalin, Potassium, permangante, Dylox, Bromex

Diseases Caused by Helminths

The term helminth includes all parasites classified as:		
	Trematoda, Cestoda Nematoda, Acanthocephala	Larvae of these parasites are found in flesh or viscera
	Adult Cestoda, Acanthocephala, Nematoda	May inhabit the lumen of the intestinal tract.
Helminths are not transmitted directly from fish to fish unless the intermediate host is another fish consumed by predator fish. As intermediate hosts serve: birds, snails and crustaceas.		1. Avoid presence of intermediate host 2. Treat helminths in intestines with: a. Santonin b. Di-n-butyl tin oxide c. Kamala

TABLE C-4 (continued)

Diseases Caused by Crustaceans (Copepods)

Crustaceans are common, bothersom, and difficult to control fish parasites. The most common are:

Argulus, *Ergasilus*,
Lernea, *Achtheres*,
Salmincola

There are freshwater and marine crustaceans. Some non-parasitic are intermediate hosts for helminths. Some copepoda bury deep in the flesh and cannot be affected by chemicals.	1. Effective control chemicals are insecticides: Bromex and Dylox added to water 2. Avoidance. Select fish free from crustacean parasites 3. Lesions may serve as portals of entry for bacteria and viruses 4. Crustaceans can transmit agents of disease from fish to fish

Miscellaneous Diseases

Fishes are subject to many neoplastic diseases, to nutritional diseases, and affected by sunlight, ultraviolet light, and fluorescent light, and often suffer from gas embolism if water is supersaturated with nitrogen or oxygen. Rapid changes in temperature are dangerous. Handling resulting in abrasions, loss of scales, or mucus, or exposure to air may be either directly lethal or make the fish more susceptible to infectious diseases.

TABLE C-5 CHEMICALS USED MOST FREQUENTLY FOR CONTROL OF INFECTIOUS DISEASES OF FISHES

Acetic acid, glacial	Dilute in water: 1:500 for 30-60 seconds (dip) 1:2000 (500 ppm) as bath for 30 minutes.
Acinitrazole (2-Acetamido-5-nitrothiazole)	Used for Hexamitiasis in Norway 50 mg per kg feed for 4 days.
Acriflavine (Trypaflavine)	5-10 ppm added to water from several hours to several days.
Avivet$^{(R)}$ (Soluble powder contains 6.6% Nifurprazine HCl (same as Carofur))	See Nifurprazine
Aureomycin$_{(R)}$	See Chlortetracycline
Betadine$^{(R)}$ (Iodophor containing 1.0% of iodine)	See Iodophors
Bithionol (2,2'-Thiobis (4,6-dichlorophenol) A French product also known as Cogla$^{(R)}$)	Orally 0.2 g/kg of fish or 2% in food. Feed for 2-3 days. For *Acanthocephala* and oral prophylaxis against *Saprolegnia*
Brilliant green (The same as Malachite green G sulfate)	See Malachite green
Bromex$^{(R)}$ (Dibrom, Naled; a pesticide)	0.12 ppm added to (pond) water for indefinite time.
Calcium cyanamide	Distributed on the bottom and banks of drained but wet ponds at a rate of 200 g/m^2.
Calcium oxide (quicklime)	Distributed on the bottom and banks of drained but wet ponds at a rate of 200 g/m^2.
Carbarsone oxide	Mixed with food at a rate of 0.2%. Feeding for 3 days.
Carofur$^{(R)}$ (A product containing 6.66% of Nifurprazine HCl.)	See Nifurprazine

TABLE C-5 (continued)

Chloramine - I	In water with pH 7.5-8.0, 18-20 ppm. Change 50% of water once each week if water temperature 10 C or below. At 25 C one treatment for 2-3 days.
Chloramphenicol (Chloromycetin$^{(R)}$)	1. Orally with food 50-75 mg/kg body weight/day for 5/10 days. 2. Single intraperitoneal injection soluble form 10-30 mg/kg. 3. Added to water 10-50 ppm for indefinite time as needed.
Chlorophos	See Dylox
Chlortetracycline (Aureomycin$^{(R)}$)	10-20 ppm in water. In eel diseases in Japan is added to feed at a rate of 10-20 mg/kg of food.
Ciodrin$^{(R)}$ (Shell Petroleum product; a pesticide similar to Dipterex)	For control of Lernea in Japanese eel culture
Cogla$^{(R)}$) (D^2N Cogla)	See Bithionol
Concurat$^{(R)}$ (2,3,5,6, tetrahydro-6-phenylimidazo (2,1-b) thiazolhydrochloride	Broad spectrum anthelmintic
Copper sulphate (Blue stone) $CuSO_4$ anhydrous $CuSO_4$ $5H_2O$ crystalline	For 1 minute dip: 1:2000 (500 ppm) in hard water add 1 ml glacial acetic acid/liter. 0.25-2 ppm to ponds. Quantity depends on hardness of water. Hard water requires more.
Cutrine$^{(R)}$ (Chelated copper compound)	Aquatic herbicide; less toxic to fish than copper sulfate.
Cyzine$^{(R)}$ (Enheptin-A)	20 ppm in feed for 3 days for Hexamita
Detrapan$^{(R)}$ (In use in France)	Systemic antifungal drug, for fish after spawning: 0.25 ml/kg intramuscularly used twice every 48 hours.
Devermin$^{(R)}$	0.1 g per kg of fish orally with food for control of Cestoda.

TABLE C-5 (continued)

Diibutylin Dilaurate (Butynorate$^{(R)}$, Tinostat$^{(R)}$)	250 mg/kg of fish orally or 0.3% in food.
Dimenton$^{(R)}$	See Sulfamonomethoxine
Dimetridazole	0.15% mixed with food for 3 days.
Dipterex$^{(R)}$	See Dylox
Diquat$^{(R)}$ (patented herbicide, Ortho Co. contains 35.3% of active compound)	1-2 ppm of Diquat cation, or 8.4 ppm as purchased added to water. Treatment for 30-60 minutes. Activity much reduced in turbid water.
Dylox$^{(R)}$ (Dipterex, Neguvon Chlorophos, Trichlorfon Foschlor, Masoten)	0.25 ppm to water in aquaria and 0.25-1.0 ppm in ponds for indefinite period.
Enheptin$^{(R)}$ (2-Amino-5-nitrothiazole)	0.2% in food for 3 days for Hexamita
Formalin (37% by weight of formaldehyde in water. Usually contains 12-15% Methanol)	1:500 for 15 minute dip 1:4000-1:6000 for one hour, 15-20 ppm to pond or aquarium water for indefinite period.
Formalin with Malachite green	Formalin, 15-25 ppm Malachite green, 0.05-1.0 ppm. For 6 hours in aquaria; may be repeated as needed. For indefinite period in ponds.
Fosfomicina$^{(R)}$ (A Spanish antibiotic; $C_3H_7O_4P$)	For *Aeromonas* infections in fishes
Furanace (P-7138)$^{(R)}$ (Ni-furpirinol); 6-hydroxymethyll-2-pyridine)	Used as bath but may be added to food. As bath: 1 ppm for 5-10 minutes; 0.05-0.1 ppm may be added for indefinite period to water. Orally for treatment: 2-4 mg/kg fish/day for 3-5 days. Orally for prophylaxis: 0.4-0.8 mg/kg fish/day as long as needed.

TABLE C-5 (continued)

Furazolidone (Furoxone N.F. 180 N.F. 180 Hess & Clark commercial products contain furazolidone mixed with inert materials)	On the basis of pure drug activity; 25-75 mg/kg body weight/day up to 20 days orally with food.
Furoxone$^{(R)}$	See Furazolidone
Furpyridinol$^{(R)}$ (product containing 10% Furanace)	Added to water 0.3-10 ppm, as is, for 30-60 minutes. Also see Furanace.
Hyamine$^{(R)}$ (Rohm & Haas Co., Quaternary ammonium germicide available as crystals or as 50% solution)	1.0-2.0 ppm (on basis of 100% product) in water for 1 hour.
Iodine	In form of a Lugol solution or iobide for control of goiter and possibly corynebacterial kidney disease.
Iodophors (Betadine, Wescodyne, Bridine, etc.)	Different commercially available iodophors contain different concentrations of iodine. To be used on a basis of pure iodine present in the product. Use 50-200 ppm iodine (usually 100 ppm) for disinfection of eggs for 10-15 minutes. Toxic to hatched fish. Probably also assists in control of some virus fish diseases.
Kamala	Mixed with diet at a rate of 2%. Feeding to starved fish for 3 days.
Kanamycin (antibiotic also traded as Cantrex, Kamycin, Resistomycin)	50 mg/1 kg of fish or 25-100 mg/kg of food. Feed for a week.
Malachite green	1:15,000 in water as a dip for 10-30 seconds, 1-5 ppm in water for 1 hour (most often used as 5 ppm). 0.05 to 2.00 ppm in ponds or aquaria for indefinite time.
Malachite green with formalin	See Formalin
Masoten	See Dylox

TABLE C-5 (continued)

Mefarol$^{(R)}$ (Probably similar to Hyamine)	1-2 ppm in water for 1 hour. Toxic in very soft water; less effective in hard water.
Methylene blue	1.0-3.0 ppm in water for 3-5 days.
Metronidazole (1-beta(hydroxyethyll) -2-methyl-5-nitroimidazole)	4 mg per liter of water for 3-4 days for control of protozoan ectoparasite in ornamental fishes.
Neguvon$^{(R)}$	See Dylox
Ni-furpirinol	See Furanace (P-7138)
Nifurprazine (HB-115) (Nitrofuran, unstable in prolonged exposure to sunlight. 1-(5 nitro-2-furyl) -2-(6-amino-3-pyridazyl) ethylene-hydrochloride) (Carofur and Aivet are water soluble formulations)	As bath: for indefinite period 0.01-0.1 ppm. In food: 10 mg/kg of food. Feeding for 3-6 days at a time.
Nitrofurazone (5-Nitro-2-furaldehyde semicarbazone)	See Furazolidone, Furoxone
Oxolinic acid (1-ethyl-1, dihydro-6, 7-methyl-enedioxy-4-oxo-3-quinoline carboxylic acid)	For control of Aeromonas infections. Orally 3 mg/kg fish once daily for 5 days. As bath: 1 ppm for 24 hours for columnaris disease.
Oxytetracycline (Terramycin$^{(R)}$)	50-75 mg/kg body weight/day for 10 days with food. (Law requires that it must be discontinued for 21 days before fish are killed for human consumption)
Ozone	Is being investigated as remedy for external infections and for decontamination of water.
Potassium permanganate $K Mn O_4$	1:1000 (1000 ppm) for a 10-40 second dip. dip. 10 ppm up to 30 minutes. 3-5 ppm added to aquarium or pond water for indefinite time.
Potential sulfonomid (Sulfadimethoxine potentiated with ormetoprim)	For control of furunculosis and other systemic infections. Used with feed 50 mg/kg fish per day.

TABLE C-5 (continued)

Povidone-Iodine (PVP-I)	See Iodophors
Quinine hydrochloride or Quinine sulfate	10-15 ppm in water for indefinite time.
Roccal$^{(R)}$ (Sold as 10-50% solution of Benzalkonium Chloride. Quaternary ammonia germicide--also see Hyamine)	1-2 ppm in water for 1 hour. Toxic in soft water; less effective and less toxic in hard water.
Sodium chloride (table salt, iodized or not)	1-3% in water from 30 minutes to 2 hours only for freshwater fishes.
Sulfadimethoxine sodium (In Japan available as powder)	100-200 mg calculated as pure drug/kg of food.
Sulfamerazine	200 mg/kg body weight/day with food for 14 days. (Law requires that treatment must be stopped for 21 days before fishes are killed for human consumption).
Sulfamethazine	100-200 mg/kg body weight/day depending on the type of food with which it is mixed. For prophylaxis reduce the quantity to 2 g/kg/day. Length of treatment as recommended.
Sulfamonomethoxine (trade name Dimeton$^{(R)}$; water soluble)	With feed as is at a rate of 100-200 mg/kg of feed. Use as needed.
Sulfisoxazole (Gantrisin$^{(R)}$)	200 mg/kg body weight/day with food
Terramycin$^{(R)}$	See Oxytetracycline
Tetrafinol$^{(R)}$	For control of intestinal helminths; used with feed.
Tin oxide, di-n-butyl	25 mg/kg body weight/day with food for 3 days.
Wescodyne$^{(R)}$ Iodophor containing 1.6% of iodine	Use as explained under Iodophors

13. GENERAL PRINCIPLES OF TREATMENT OF SYSTEMIC BACTERIAL DISEASES OF FISHES

by

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(1975)

Treatment of systemic bacterial diseases of fishes with drugs (chemotherapy) started in the forties when Dr. Gusell reported on the successful use of sulfamerazine and nitrofurazone (Furacin^R) for the treatment of furunculosis in trout. Most of the work carried out in the fifties was performed at the Eastern Fish Disease Laboratory by myself and associates, and Schaperclaus in Germany. More recently, research on control of diseases of pacific salmon, catfish, and in fresh and saltwater aquaculture have been carried out at the Western Fish Disease Laboratory by Dr. Rucker and his associates; by Dr. Meyer at Stuttgart, Arkansas; and by the Japanese investigators. Resently Schaperclaus of Germany introduced a mixed treatment of chloramphenicol and immunization for control of _Aeromonas_ infections. Dr. George Post and his associates concentrated on the use of furazolidone (Furoxone^R) for furunculosis in trout.

In research at Leetown our guidelines were as follows:

1. The selected drug must be effective in control of diseases of humans and domestic animals.
2. Must be available in a less expensive veterinary formulation.
3. Must be effective if given orally with food, or added to water.

We were successful here at Leetows in furnishing sufficient data to the FDA for the clearance of sulfamerazine for the treatment of furunculosis and oxytetracycline (Terramycin^{R}, Pfitzer) for *Aeromonas* infections and ulcer disease (Hemophilus piscium) in catfish and salmonids. Clearance of Terramycin was a joint effort of the laboratories at Leetown, West Virginia, Seattle Washington, and Stuttgart, Arkansas.

The Japancese investigators made an important contribution by the introduction of Ni-furpirinol (Furanace^{R}), a nitrofuran which the fish absorb from the water, for control of several systemic bacterial infections.

We here at Leetown, and Schaperclaus in Germany, introduced chloramphenicol as an excellent antibiotic for control of *Aeromonas* in bacterial diseases of fishes. In the USA the use of chloramphenicol for control of any disease, except very severe diseases of humans, is opposed in order to prevent the appearance of pathogens resistant to this drug. Wide presence of such pathogens would make this "last report" antibiotic much less dependable.

In Europe chloramphenicol is used often for the treatment of diseases of fishes and is less expensive than here.

The dosage of themotherepeutic drugs should be as high as safety permits because it is important to stop the disease fast -- before drug resistant forms of bacteria develop. Therefore, salfadrugs are given to fish in food at a rate of 200 mg/kg body weight/day. With some diets, 100 mg is sufficient.

Nitrofurans such as Furoxone^{R} are recommended at a rate of 75 mg/kg per day with food. Lower dosages are also used.

Furanace^{R} is added to water at a rate of 1 ppm for treatment from 10 minutes up to 1 hour, and 0.5-0.2 ppm for indefinite period.

Antibiotics such as oxytetracycline and chloramphenicol are used orally with food at a rate of 50 to 75 mg/kg fish/day. Use of antibiotics in soluble form added to water in aquaria varies from 10 to 50 mg/liter. This dosage is not based on critical research, but is believed to be satisfactory. Nothing definite is known, at least by me, on the effect of antibiotics and Furanace^R on the operation of the biological filtration system in aquaria. These are the principles of the treatment of systemic infections in fishes.

In external bacterial infections caused by flexibacteria (myxobacteria) such as columnaris disease and gill disease which may become systemic, the same is true. At the present time, Furanace^R added to water is the drug most often chosen.

Of the external protozoon disease infections with Oodinium (velvet disease), chlortetracycline (Aureomycin^R) added to water at a rate of 10 to 20 mg/liter is believed to be satisfactory. Other external disinfectants are also good in this and other external infections with protozoons.

Treatment of diseases of fishes in closed containers with water which is seldom exchanged or which is recirculated presents various difficulties. If drugs are added to water in such concentrations that pathogenic bacteria are killed, they may be toxic to fishes and plants, and are very likely to adversely affect biologically active filters. If concentration is so low that the drug acts only in a bacteriostatic way, then the bacteria are not eliminated and may easily produce drug resistant, or even drug dependent variants. When the drug decomposes, or its concentration becomes lower, the pathogens can again multiply.

In systemic chemotherapy antibiotics usually act as bacteriostats. If the conditions which led to an outbreak of a disease is the first place are not removed, the recurrence of the disease is likely. Drugs can be compared to fire extinguishers, which may extinguish a fire if it did not progress too far, but will not prevent future fires.

I do not believe that the shotgun method of treatments without correct diagnosis and selection of the proper drug has value for the average aquarist. However, well selected and correctly applied treatments may be of great value for fish growers, shippers and dealers, provided they have the necessary knowledge and experience, as well as proper diagnostic facilities. The same is true with those hobbyists who have the proper biological training and experience.

References

Regarding control of diseases of fishes, I should like to recommend the review paper by Dr. R.L. Herman, "Prevention and Control of Fish Diseases in Hatcheries", Symposium on Diseases of Fishes and Shellfishes, Special Publication No. 5 of American Fishery Society, Pages 3-15. I also suggest the tables by Dr. Snieszko listing drugs and treatments which are frequently updated. These tables are available on request from the Eastern Fish Disease Laboratory.

Enclosed is also FDL-38 by Dr. Wedemeyer which speaks for itself.

14. AUTOPSY PROCEDURE

Fish specimens collected should be autopsied as soon as possible. Only live specimens or those which have been dead less than a few hours are suitable. The pathologist should have ready the following equipment and supplies:

Dissecting tools:

- 2 scalpels with blades #12 and #22
- Fine pointed and sturdy dissecting scissors
- Bone cutters
- Fine pointed and sturdy forceps
- Hemostatic forceps
- Teasing needle

Autopsy trays
Iodophor (diluted 1:5)
Clean slides and coverslips
70% ethanol for flaming
Plastic bags for disposing of fish
Giemsa stain and buffer, absolute methanol
Washtub of Iodophor to soak large fish
Physiological saline
Pathology forms

Media:

Freshwater fish
BHIA plates
Plain Cytophaga plates for myxobacteria on gills and kidney
Rimler-Shotts small plates for Aeromonas hydrophila
TCBS agar plates

Thioglycollate tubes
Mueller-Hinton medium with co-carboxylase for
Haemophilus piscium
Sabouraud-Dextrose medium for fungi
Lowenstein-Jensen slants for mycobacteria

Marine fish

BHIA (marine) plates
Seawater cytophaga
Rimler-Shotts
TCBS agar plates
Thioglycollate tubes
Sabouraud-Dextrose medium for fungi
Lowenstein-Jensen slants for mycobacteria

The following procedure should be used as a guideline for fish autopsy:

Blood sample - Withdraw blood from caudal sinus with a heparinized syringe. Place a small drop on a cleaned, alcohol-washed slide. With the edge of another slide held at a 45° angle, smear the blood over the entire slide with one motion. Let dry. Fix in absolute methanol 10 minutes. Let dry. Stain with Giemsa stain as per directions.

Kill fish with several sharp blows between the eyes or anesthesize with 10% benzocaine (2 ml/gal.)

Skin - Scrape skin with a large scalpel blade to include some tissue. Put a few drops of saline on a slide and mix in the skin scrapings. Examine at 10X and 40X for external parasites. Culture any skin lesions for bacteria and fungi.

Gills - Look for parasites on one side: Cut off operculum. Cut off one gill filament and cut off the gill raker if necessary. Place on a slide with saline. Look for protozoans as well as for clubbing, fusion, or hypertrophy of lamellae. Use the other side for bacteriology: Flame forceps and pull up operculum. Sterilize loop, go into lamellae without touching sides, and wiggle loop to obtain noncauterized tissue. Culture on media (Table 1).

Internal Organs - Disinfect whole fish in Iodophor bath for five minutes. Flood tray and fish with Iodophor. Secure fish by throat with flamed forceps. Use #12 scalpel blade (flamed twice) to slit just through skin from shoulder girdle to anus with caution so as to not cut into viscera. Cut off abdominal wall or hold flap open with hemostats. Take organ samples for plate culture (Table 1).

Sample organs for microscopic examination: Place a bit of organ tissue on a slide and tease apart in saline. Examine for parasites and bacteria. Then allow the slide to dry and Gram stain for bacterial examination.

If further analysis of fish tissue is required, samples are prepared in the following manner:

Virology - Liver, kidney, and spleen from each fish are pooled, placed in a sterile screw cap tube, and frozen for delivery to Dr. Sigel.

Histology - Organs or tissues are fixed in buffered-formalin saline (Appendix III). Brain is fixed in formalin-ammonium bromide. Tumors are fixed in both Bouin's and buffered-formalin saline (Dr. Haines).

Pesticides - Liver, brain, and/or muscle is placed in heat or acid cleaned test tubes with teflon lined caps and refrigerated until distribution.

Heavy metals - Liver or muscle is placed in plastic snap-top vials and refrigerated until distribution.

The pathology form is filled in as the autopsy proceeds. Tag number of the fish, species, date, and person performing the autopsy should be recorded on the form. A summary of conclusions should appear at the end of the form.

Table 1. Scheme depicting media to be utilized in culturing organ systems for pathogenic bacteria in dead and moribund freshwater (FW) and marine (MAR) fish.

	BHIA		Cytophaga		TCBS		Thioglycollate		Rimler-Shotts		Mueller-Hinton w/ co-carboxylase		Lowenstein-Jensen		Sabouraud-Dextrose	
	FW	Mar	FW	Mar	FW	Mar	FW	Mar	FW	Mar	FW	Mar	FW	Mar	FW	Mar
Skin lesions	✓	✓	✓	✓	✓	✓			✓	✓	✓		✓	✓	✓	✓
Eyes	✓	✓			✓	✓										
Gills	✓	✓	✓	✓											✓	✓
Liver	✓	✓			✓	✓			✓	✓	✓		✓	✓	✓	✓
Spleen	✓	✓														
Kidney	✓	✓	✓	✓	✓	✓			✓	✓	✓		✓	✓	✓	✓
Brain	✓	✓					✓	✓								

SAMPLING PROCEDURE OUTLINE
FOR
SOUTHEAST FLORIDA OCEAN OUTFALL STUDY
EXPERIMENTAL FACILITY

**DILUTION WATER
(Control Tanks)**

Chemistry

Nutrients
(Tony Mendez)

Toxicology
(Eugene Corcoran)

RAW AND PROCESSED SEWAGE

Nutrients, etc.
(Shaukat Farooq)

EXPERIMENTAL TREATMENTS AND DILUTIONS IN TANKS
(6X3)

Parameter	Frequency
(1) Dissolved oxygen (DO) Maintain over 70% saturation YSI Oxygen Meter	1 X 24h
(2) Temperature	Continuous
(3) Salinity (conductivity) YSI Salinometer and Refractometer	2 X 24h
(4) Residual chlorine Amperometric titration	2 X 24h
(5) NH_3 - H_2S - pH	per prelininary experiment
(6) MBAS (methylene blue active substances)	per preliminary experiment
(7) Suspended solids	1 / 2 days
(8) Bacteriology a. total Vibrios (TCBS) b. total heterotrophs	1 / week
(9) Virology (Tim Edmond)	per preliminary experiment

a. Detection and typing of enteric virus
    - total coliform
    - fecal coliform
    - fecal Streptococci
    - bacteriophage

b. Viral survival

c. Viral adsorbtion to particulates in sediments

(10) Nutrients and toxicology of treatments (not dilutions)
T. Mendez and E. Corcoran

EXPERIMENTAL FISH

A. Growth and Pathology

Initial

(1) Weight

(2) Length

(3) Note unusual items, as scale disorientation

(4) Toxicants - take pooled, representative samples (see ASTM)

(5) Toxicants in diet

Dead

(1) Weight and length where applicable

(2) Autopsy:
- external symptoms
- bacteriology
- selected virology
- histology where applicable

(3) Selected toxicology

Survivors

(1) Weight

(2) Length

(3) External symptoms

(4) Selected bacteriology

(5) Selected virology

(6) Selected autopsy

(7) Selected histology

(8) Toxicology of pooled samples

B. Behavioral Changes (Art Myrberg)

(1) Respiration difficulties

(2) Lack of flight response

(3) Alteration of feeding habits
- surpressed feeding
- trailing feces

APPENDIX D

METHODS OF CHEMICAL ANALYSIS

CONTENTS

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GENERAL PROCEDURES

The parameters analyzed during the SEFOOS bioeffects experiments included basic sewage treatment plant operating criteria, major nutrients, metals of toxic importance, and chlorinated hydrocarbons which included both pesticides and polychlorinated biphenyls (PCB's) and phthalic acid eaters (PAE's). Table D-1 lists the parameters analyzed by compartment.

The analyses were performed on wastewaters and the organisms exposed to the diluted effluents. The untreated influent and processed wastewaters were analyzed for operational parameters, nutrients, metals, chlorinated hydrocarbons and PAE's. Coral tissues were analyzed for nutrients, metals and chlorinated hydrocarbons. Fish and shrimp were analyzed for metals and chlorinated hydrocarbons.

CLEAN PROCEDURES

Great care was taken in the collection, storage, laboratory extraction/concentration, and analysis of all samples to insure that no contamination was introduced and the parameter of interest was preserved. All sample containers, collection devices,

TABLE D-1. ANALYTICAL PARAMETERS

Operational Parameters	Nutrients	Metals	Chlorinated Hydrocarbons	Phthalic Acid Esters
Residue	Ammonia	Cadmium	Aldrin	Di-ethylhexyl
Total	Nitrite	Chromium	Heptachlor	Di-isobutyl
Nonfiltrable	Nitrate	Copper	Epoxide	Di-butyl
pH	Ortho	Lead	o,p'-DDE	Butylglycolbutyl
Biochemical	Phosphate	Zinc	p,p'-DDE	Butytlbenzyl
Oxygen Demand			Dieldrin	
Turbidity			o,p'-DDD	
Organic Carbon			p,p'-DDD	
			Endin	
			o,p'-DDT	
			p,p'DDT	
			Aroclor 1254	
			Aroclor 1016	

laboratory glassware and storage containers were cleaned in the appropriate manner for the sample collected or being processed.

Containers and apparatus coming in contact with the nutrient and sewage treatment plant operation evaluation samples were cleaned with a hot water and soap wash, rinsed twice with tap water and twice with distilled water. Samples for metal analysis were handled only by containers and utensils, which had been washed and rinsed in the above described manner, rinsed with concentrated nitric acid and then with distilled-deionized water. Care was taken to insure that no metal came in contact with the sample. All collection and storage containers were constructed of conventional polyethylene as recommended by Patterson and Settle (1976). Chlorinated hydrocarbon and PAE samples were handled with only glass, teflon or metal utensils. Glass storage and collection containers were washed in the same manner as described for the nutrients and burned at 500°C in a kiln to remove any traces of organic contamination. Metal and teflon items were subjected to repetitive hexane rinses. Care was taken to insure that no plastic or organic contaminants came in contact with the samples.

COLLECTION METHOD

A standardized self-sticking label was developed for all the wastewater collections. The label contained the following information:

SEFOOS-STABEL

Analysis Sample Type:
Location: Time:
Date:
Collection Method:
Preservation Used: Quantity:
Collected By:
Sample # Laboratory #

This label was completely filled out and attached to all samples collected.

Operational samples for the evaluation of the sewage treatment plant were collected on alternating days, as grab samples from the primary, secondary, chemical flocculation effluents, incoming untreated wastewater and return activated sludge processors. The grab samples represented single sampled portions and were collected at a time most representative of the parameters of interest. The collection time was always during the late morning to early afternoon. This represented a portion of the daily high flow period experienced by the Virginia Key Sewage Treatment Plant. The samples were collected in conventional polyethylene 1-liter containers. Immediately after collection, the samples were stored at 0°C until analyzed.

The wastewater samples for nutrient analysis were collected in the same manner and at the same time as the sewage treatment plant operating parameters. They were collected in 1-liter conventional polyethylene bottles from the primary, secondary, chemical flocculation effluents and the untreated wastewater. The samples were immediately returned to the laboratory, subsampled and diluted 50x with distilled-deionized water. A portion from the diluted sample was transferred to a 150-ml plastic bottle, given a code number, and transported to the Rosenstiel School of Marine and Atmospheric Science's Water Analysis Laboratory, for the determination of nitrite, nitrate, and ortho phosphate by auto-analyzer. If analysis could not take place immediately, the samples were stored at 4°C by the Water Analysis Laboratory personnel. Both the remaining undiluted and diluted samples were archived in a refrigerated storage locker at 4°C until the results from the analyses had been completed and evaluated as satisfactory.

Samples of wastewaters for metal analysis were collected by an auto-sampler (described in detail below) from the primary, secondary, chemical flocculation effluents and the incoming untreated wastewater. The samples were daily composites collected in 1.8-liter conventional polyethylene sample bottles containing concentrated nitric acid as a preservative and stored at 4°C. These daily composites were then combined into equally weighted (by volume) weekly composites. The weekly composites were stored at 4°C until analyzed.

Chlorinated hydrocarbons and PAE's were also collected by the auto-samplers from the same treatment processes as the metals. They were collected in glass jars with teflon-lined plastic caps, stored at 4°C until the weekly composites could be completed and analysis performed. The weekly composites were handled in the same manner as the metals.

Shrimp and fish samples were collected from the bioeffects tanks for metal and chlorinated hydrocarbon analysis. Each sample for metal analysis was secured in an inner acid-washed, heat-sealed plastic bag which was enclosed by another heat-sealed plastic bag containing the coded sample number. Organism samples for chlorinated hydrocarbon analysis were first wrapped in aluminum foil, and then secured within two heat-sealed plastic bags. The outer bag contained the sample code number.

Coral tissue was removed from its skeleton by a high pressure water jet. The water and tissue mixture was then homogenized with a Waring blender and subsampled for the various analyses. All tissue samples (coral, fish and shrimp) supplied as blind samples and identified by coded number only.

Auto-Samplers

Four prototype discrete sequential automatic samplers were designed and built by Sigmamotor, Inc., 14 Elizabeth Street, Middleport, New York 14105 (phone 716/735-3616) for the project. The Sigmamotor Model 6301 Special Discrete 8 Sample Sequential Automatic Samplers were designed to collect a representative wastewater sample for both the metal, chlorinated hydrocarbon and PAE analysis. The refrigerated units collected an adjustable volume of sample at given intervals and distributed it equally into two separate sample containers, one for metals and the other for chlorinated hydrocarbons and PAE's.

The sampler can be set to automatically collect samples at a preset timed interval, either 1-99 minutes or hours, or at interval controlled or paced by an external source (i.e., flow measuring device). The sample volume was supplied to the unit by a positive displacement roller pump (peristaltic type) with an adjustable pumping duration of 1-99 seconds. Purging of the sample collection line was accomplished by a 20-second air pre-purge cycle before each sample was taken and an adjustable, 10-90 second, air or liquid purge after collection. The air/liquid purge was controlled by an electrically activated, teflon solenoid valve system. A multiplex control unit was provided that allows 1 to 8 consecutive samples to be deposited into a single bottle in the SAMPLES PER BOTTLE mode or 1 to 8 consecutive bottles per sample interval in the BOTTLES PER SAMPLE mode. A third mode was available, COMPOSITE, in which a single large composite sample maybe collected.

The sequence of operation for the auto-samplers was as follows. After the unit was initialized, the pump started in reverse direction for a preset period of 20 seconds purging the sample line with atmospheric air. Next, the unit activated the pump drawing a sample for a predetermined time (function of volume needed); this then was followed by the final pump reversal for the liquid or air purge. The unit could either advance to the next pair of bottles or continue sampling into the present pair of bottles until the selected number of composites had been completed, then it advanced to the next pair of bottles. The unit continued until the sampling sequence had been completed, then it automatically terminated operation and a red indicator light labeled SAMPLING COMPLETE came on and remained lit until the unit was reset.

The unit was designed to minimize sample contamination as much as possible within budgetary constraints. The collection tubing from the sample source to the roller pump was 6.35 mm ID teflon. The roller pump was equipped with Dow Corning 601-565, 9.38 mm ID, Medical Grade Silicon tubing. After leaving the silicon tubing, the sample again was transported by 6.35 mm ID teflon tubing into the refrigerated housing. It was then deposited into a splitter distribution unit constructed of 12.70 mm ID food processing grade plastic piping and from there it gravity flowed into the paired collection containers.

For the purpose of this project, the sampler was operated in the SAMPLES PER BOTTLE mode. The multiplexer was set to 8 and the pumping duration was set to allow a volume of 188 ml to be pumped into each of the paired sample bottles every three hours. This generated a pair of sample bottles each containing approximately 1.5 liters of sample every 24 hours. The collection tray was loaded with eight 1.8-liter sample bottles, four glass (chlorinated hydrocarbons and PAE's) and four conventional polyethylene (metals). Nitric acid was added to the polyethylene bottles as a preservative just before being loaded into the samplers. The unit was able to operate four days unattended taking four paired composite samples consisting of eight discrete time periods during each day.

Tests were run to determine the accuracy of the sample splitting unit. Each of the four sampling units were run through two complete cycles. After each complete composite, the volume in each paired sample was determined. The mean of the percent difference between all of the paired samples was 19 with a standard deviation of 15. These values were acceptable under the sample protocol of the project. Later during the project it was discovered that the difference in the splitting efficiency could be improved by leveling the splitter/distributor arm for its complete rotation.

QUALITY CONTROL

Blanks, replicates, U.S. EPA Quality Assurance and National Bureau of Standards samples were incorporated in each sample run as appropriate. A minimum of 10 percent of each sample run for nutrients and metals were quality control samples. U.S. EPA Quality Assurance samples were used where available for the treatment plant operational samples.

ANALYTICAL PROCEDURES

The methods described in Methods for Chemical Analysis of Water and Wastes (U.S. EPA, 1975) were used to determine all the operational characteristics of the sewage treatment plant. The method numbers referred to in the text are from this publication.

Sewage Treatment Plant Operating Parameters

These samples were immediately frozen upon collection and stored until analysis could be done. The samples were analyzed for residue, both total (total, fixed and volatile) and nonfilterable (total, fixed and volatile), pH, biochemical oxygen demand (BOD), turbidity and organic carbon.

Total residue was determined by the gravimetric procedure, Method 160.3. Volatile total residue was determined by igniting the total residue at 550°C as described in Method 160.4. Total fixed residue was determine by the difference in the two fractions (total residue - total volatile reidue = total fixed residue). Non-filterable residue was determined gravimetrically after filtering the sample through a glass fiber filter (Reeve Angle 935-AH) and drying (Method 160.2). The volatile and fixed fractions were determined in the same manner for the total residue.

The pH of the samples were determined electrometrically with an Orion Model 407A Specific Ion Meter using a glass combination electrode. The procedure followed is described in Method 150.1.

The biochemical oxygen demand (BOD) was determined using Method 405.1. This involves determining the initial oxygen value for a wastewater sample, inoculating the sample with a nutrient mixture and incubating the sample for five days at 20°C. The reduction in the oxygen content during the incubation period yields an estimate of the BOD. All samples analyzed for BOD were diluted when appropriate with distilled-deionized water and each sample was run in triplicate. The oxygen concentration was determined by a YSI Model 51B Oxygen Meter equipped with a self-stirring BOD probe.

Turbidity was determined using the nephelometric method (Method 180.1). This method is based on the comparison of the intensity of light scattered by the sample with a standard reference material. The Hach Laboratory Turbidimeter, Model 2100A was used for all determinations. All samples, when appropriate, were diluted with distilled-deionized water and values were reported in NTU's (nephelometric turbidity units).

Organic carbon was determined on the unfiltered wastewaters and coral tissues by converting the carbon to CO_2 by catalytic combustion and measuring it directly by an infrared detector. This procedure follows that outlined in Method 415.1. Removal of the inorganic carbon from the sample consisted of a pretreatment process. The wastewater sample was acidified to a pH <2 and a high purity nitrogen gas was bubbled through it (Van Hall et al., 1963). The acidification converted all of the inorganic carbon to CO_2 and the bubbling process facilitated its removal. The organic carbon was determined using Beckman Model 915 Total Organic Carbon Analyzer.

Nutrients

The nutrients analyzed for in the wastewater and coral tissue were ammonia, nitrate, nitrite, and ortho phosphate. All samples analyzed were nonfiltered and diluted 50x with distilled-deionized water prior to analysis.

Ammonia was determined by the phenol-hypochlorite method described by Strickland and Parsons (1972). All samples were analyzed immediately after collection. The blue indophenol color formed with ammonia is porportional to its concentration and was measured on a Beckman DU-2 Spectrophotometer.

Nitrate, nitrite and ortho phosphate were determined colorimetrically by a Technicon Model CSM-6 Continuous Simultaneous Multi-Channel Auto Analyzer. The methods used for the determinations were originally developed for the analysis of seawater by auto-analyzer but parallel those known accepted and used for wastewaters in U.S. EPA (1979).

Nitrite was determined by reacting the nitrite ion with sulfanilamide under acid conditions to form a diazo compound. This compound when coupled with N-napthylethylenediamine forms a reddish-purple color porportional to the concentration of nitrite (Armstrong et al., 1967). This procedure follows Method 354.1.

Nitrate + nitrite was determined by the procedure in which nitrate is reduced to nitrite by a copper-cadmium reduction column (Armstrong et al., 1967). The nitrite is then determined by the same method described above for nitrite. This method is the same as the colorimetric, automated cadmium reduction procedure described in Method 353.2.

Ortho phosphate was determined by using the single reagent method (Murphy and Riley, 1962). This procedure uses a phosphomolybdenum blue complex which is proportional to the concentration of ortho phosphate in the sample. This method is basically the same procedure reviewed in detail by Method 365.1.

Metals

Wastewater and coral tissue samples were analyzed for total metals by digestion in concentrated nitric acid using a procedure, with selected modifications, that followed the one suggested in U.S. EPA (1979). A known volume of sample was placed in a quartz glass boiling flask with the addition of 5 ml of concentrated nitric acid. The sample was taken to dryness using a bank of heat lamps (approximate temperature was 110°C). An additional 5 ml of concentrated nitric acid was added, the flask was covered with a quartz glass condensor and allowed to reflux for 24 hours. The sample was then diulted to a known volume with a 10 percent solution of nitric acid, transferred to a conventional polyethylene screw cap container and sent to the Division of Marine and Atmospheric Chemistry for analyses.

Flameless atomic absorption was used to determine the metal concentrations. Analysis was done using a Perkin-Elmer Model 403 Atomic Absorption Spectrophotometer. The unit was equipped with a Model HGA-2100 graphite furnace, an auto-sampler, deuterium background corrector, and both electrodeless (EDL) and hallow cathode lamps. Each sample was run in triplicate. The instrument was housed in a special room dedicated solely to trace metal analysis. The detection limits were 2 ug/l for cadmium, chromium, copper, lead and zinc.

Preparation of the tissue samples (fish and shrimp) for metal analysis followed basically the same procedure as used for wastewaters. Whole specimens (1 to 3 individuals depending on the quantity available) were placed in a preweighed quartz boiling flask. The flask plus organisms were weighed, placed in a clean drying oven and dried at 110°C to a constant weight. The flask and dried organisms were weighed and the dry weight determined. A known amount of concentrated nitric acid was added and the organisms were allowed to digest under mild heat, supplied by a bank of heat lamps. After the sample had gone into solution, an additional 10 ml of nitric acid was added and a quartz glass condensor was placed on the flask. The sample was allowed to reflux for 24 hours, cooled and then brought to a known volume with 10 percent nitric acid. The sample was analyzed by the Division of Marine and Atmospheric Chemistry.

Chlorinated Hydrocarbon and PAE Analysis

The wastewater was extracted using the same procedure developed by the Southern California Coastal Water Research Project (646 West Pacific Coast Highway, Long Beach, California 90806, phone 213/435-7071) and tissue samples were analyzed using the procedures outlined by Thompson (1977) in "Manual of Analytical Methods for the Analysis of Pesticide Residues in Human and Environmental Samples."

Wastewaters were extracted by three successive liquid-liquid extractions. A 1-liter sample was placed in a 2-liter glass separatory funnel equipped with a teflon stopcock. The first extraction consisted of the addition of 75 ml of 15 percent (volume) diethyl ether in hexane. The separatory funnel was shaken for two minutes and the layers allowed to separate. The organic layer was placed in an erlenmeyer flask containing anhydrous sodium sulfate and the aqueous layer was returned to the separatory funnel. Fifty ml of a 6 percent solution of diethyl ether in hexane was placed in the separatory funnel and it was shaken again for two minutes. The organic fraction was combined with that from the first separation. The third extraction was with 50 ml of hexane. The same procedure was followed and the organic fraction was combined with the previously extracted fractions. The ether-hexane extract was transferred to a Kuderna-Danish apparatus, placed in a steam bath and the volume reduced to approximately 5 ml.

The samples were then cleaned and fractionated by use of the Florisil column. A slurry of activated Florisil and hexane was added to a depth of 80 mm in the chromatographic column (25 mm OD, 22 mm ID, and 400 mm in length) equipped with a teflon stopcock. Ten mm of anhydrous sodium sulfate (previously burned at 500°C to remove any organic contaminants) was added on top of the Florisil. The concentrated 25 ml sample was added to the Florisil column and separated into three fractions. The column was first eluted with 50 ml of 6 percent diethyl ether in hexane (fraction 1), then with 50 ml of 15 percent diethyl ether (fraction 2) and finally with 50 ml of 50 percent diethyl ether in hexane (fraction 3). Fraction 1 contained Aldrin, Heptachlor Epoxide, o,p'-DDE, p,p'-DDE, o,p'-DDD, p,p'-DDD, o,p'-DDT, p,p'-DDT and the PCB's. Fraction 2 contained Dieldrin and Endrin and fraction 2 contained the PAE's.

Fraction 1 was further subdivided into a fraction containing only the PCB's by saponification (dehydrohalogenation). This process removed the pesticides leaving only the PCB's. About 20 ml of fraction 1 was placed into a boiling flask fitted with a standard taper ground glass joint. A mixture of 5 g of KOH dissolved in 50 ml of ethanol was added and the flask was fitted with a Snyder condensor. The mixture was placed on a steam bath and allowed to reflux for 30 minutes. The contents of the flask were placed in a separatory funnel along with 100 ml of organic free water (prepared by passing distilled water through an XAD-2 resin column), 50 ml of hexane was added and the sample was shaken for 5 minutes. The aqueous layer was discarded and the organic layer washed with two 100 ml portions of water. The saponified raction was then concentrated using a Synder condensor and steam bath.

The micro method for the determination chlorinated pesticides in human or animal tissue as described by Thompson (1977) was used for the extraction and concentration of coral, fish and shrimp tissues. This procedure was designed to be used with small quantities of tissue. Approximately 0.5 g of tissue was homogenized in a tissue grinder with acetonitrile. An aqueous solution of sodium sulfate was added to the sample, the pesticides were separated into hexane and the extract was concentrated using a micro Kuderna-danish apparatus and a steam bath. Cleanup and partitioning of the two fractions were accomplished by successive elutions with hexane and 1 percent methanol in hexane through a Florisil column topped with anhydrous sodium sulfate. Two fractions were collected, concentrated and analyzed.

The samples were analyzed with a Tracor Model 222 Gas Chromatograph. The unit was equipped with two electron capture ^{63}Ni detectors. Packed glass columns, 2.0 m long, 6 mm OD, and 2 mm ID were used. Column 1 was packed with 1.5 percent SP2250, 1.95 percent SP2401 on 100/120 mesh Gas Chrom Q and column 2 with 4 percent SE-30, 6 percent SP2401 on 100/120 mesh Supelcoport (both were prepared by Supelco, Bellefonte, Pennsylvania

16823). Column 1 was used for quantification of the compounds, while column 2 was used for verification. The carrier gas was purified nitrogen with a flow rate of 80 ml/min. The temperatures for the injectors, column and detectors were 225, 205 and 285°C, respectively.

The chlorinated hydrocarbons and PAE's were identified and quantified by comparison of retention times and peak heights of the samples with those of standards. Standards were prepared from pure compounds received from the U.S. EPA repository located at Research Triangle Park, North Carolina. Standards were analyzed with each day's sample run. The detection limits were determined by the measurement of the smallest peak that would be quantifiable under the operating conditions used during the project. Table D-2 lists the compounds quantified during the project and their detection limits.

The PCB's presented a somewhat more difficult quantification problem in that these compounds were characterized by multiple peaks and in many cases there were multiple compounds in each sample. It was determined that by far the most dominant PCB's were Aroclor 1016 and 1254 (personal communication, Dr. Eugene F. Corcoran). To assist in the isolation and quantification of these two compounds, a table (Table D-3) was constructed from standards using their relative retention ratios (Aldrin ratios) and relative peak heights (ratio of highest peak in Aroclor to all other peaks in that Aroclor). Using this table two dominant peaks, for each Aroclor, were chosen which did not overlap the other compound's retention times. These two peaks, for each Aroclor, were quantified (sample peak height compared to standard peak height) and averaged. This method produced a semi-quantitative estimate of the amounts of the two Aroclors.

CHLORINATION SYSTEM

Each of the three effluents generated by the wastewater treatment plant were chlorinated by a separate Wallace & Tiernan (Pennwalt Corporation, 25 Main Street, Belleville, New Jersey 07109) chlorination system. This unit consisted of am amperometric titration system (see Rand, 1979, Method 409 C for detailed description) that continuously sampled and analyzed the effluent for its chlorine content. The analyzer was connected to an indicator unit equipped with a meter readout which visually displayed the concentration of the chlorine and in addition was connected to an adjustable metering pump which controlled the input of chlorine into the system.

Each effluent had a separate and independent operating chlorination system which incorporated a Series A-792 Residual Chlorine Analyzing Unit For Wastewater, a Series A-780 Indicator, and an electric positioner connected to a Series 44

TABLE D-2. CHLORINATED HYDROCARBON AND PAE DETECTION LIMITS

Compounds	Detection Limits (pg)
Pesticides	
Aldrin	10
Heptachlor Epoxide	10
o,p'-DDE	10
p,p'-DDE	10
Dieldrin	10
o,p'-DDD	15
p,p'-DDD	15
Endrin	20
o,p'-DDT	20
p,p'-DDT	20
Polychlorinated biphenyls	
Aroclor 1016	200
Aroclor 1254	200
Phthalic acid esters	
Di-ethylhexyl	3000
Di-isobutyl	3000
Di-butylglycolbutyl	3000
Butylglycolbutyl	3000
Butylbenzyl	3000

TABLE D-3. PCB RETENTION RATIOS AND RESPONSE FACTORS

Aroclor 1016		Aroclor 1254	
RRR[a]	RPH[b]	RRR	RPH
0.55	0.69	0.52	0.05
0.67	0.57	0.63	0.06
0.83	1.00	0.79	0.26
1.01	0.75	0.96	0.71
1.18	0.85	1.13	0.47
1.42	0.25	1.42	0.94
		1.57	0.99
		1.95	0.71
		2.09	1.00
2.21	0.08		
		2.37	0.95
		2.63	0.51
2.95	0.01	2.98	0.60
		3.35	0.68
		4.77	0.08
		5.09	0.11

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[a] RRR is the relative retention ratio compared to Aldrin.

[b] RPH is the relative peak height response compared to the tallest peak is underlined.

metering pump. The effleunts were pumped into a chlorine contact/mixing tank in which chlorine was added and a gravity flow system continuously supplied a sample to the titrators. The amperometric titration system continuously titrated the sampled effluents and sent electronic impulses to the indicator/control unit. This unit was equipped with a visual meter display and a manual control system for setting the upper and lower limits for the chlorine concentration in the effluents. The indicator unit controlled an adjustable metering chlorine supply pump. When the chlorine concentration deviated from the accepable range set on the indicator unit, electrical pulses would be sent to the metering pumps to increase/decrease the volume of chlorine being supplied to the contact tank.

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APPENDIX E

PARTICIPANTS IN SEFOOS STUDY

The following list identifies the individuals (and, where applicable, the department of the University of Miami and Principal Investigators with whom they were associated) who participated in the SEFOOS study:

1. Rosenstiel School of Marine and Atmospheric Science (RSMAS)

Dr. Samuel C. Snedaker-Biology and Living Resources (P.I.)[a]
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    Dr. Joan Browder
    Mr. Melvin S. Brown
    Mr. Daniel Cottrell
    Ms. Ann Dunbar-Cooper
    Dr. Charles Getter
    Mr. Mark Newman
    Mr. Raoul Reher

Dr. Eugene Corcoran-Biology and Living Resources (P.I.)
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    Dr. James Carpenter
    Ms. Ana Freay
    Mr. Andy Friden
    Ms. Helene Hanchett
    Mr. Manny Master
    Mr. Tony Mendez
    Ms. Cynthia Moore
    Mr. Carroll Smith
    Mr. John VanLandingham

Dr. Dennis Taylor-Biology and Living Resources (P.I.)
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Dr. Donald Marszalek-Marine Geology and Geophysics (P.I.)
    Dr. Susan Markley

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2. School of Medicine

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      - Ms. Carmen Gomez
      - Mr. Steve Newman

   Dr. Lanny Udey-Department of Microbiology (P.I.)
      - Ms. Charlene Grall
      - Mr. Dana Kent
      - Ms. Elaine Kraus-Gordon

3. School of Engineering

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      - Ms. Shaheen Akhlaque
      - Mr. Paul Gabriel

4. Administration

   Dean William Hay - RSMAS
   Mr. George Chipouras - Facilities Planning and Construction

5. Metro-Dade Water and Sewer Authority

   Mr. Norman Pascul

6. Consultants

   H.J. Ross and Associates-Consulting Engineers
      - Mr. "Bud" Arvia, Director, Sanitary Division
      - Mr. Roger Bass, Architect
      - Mr. K.C. Kelly, Vice President

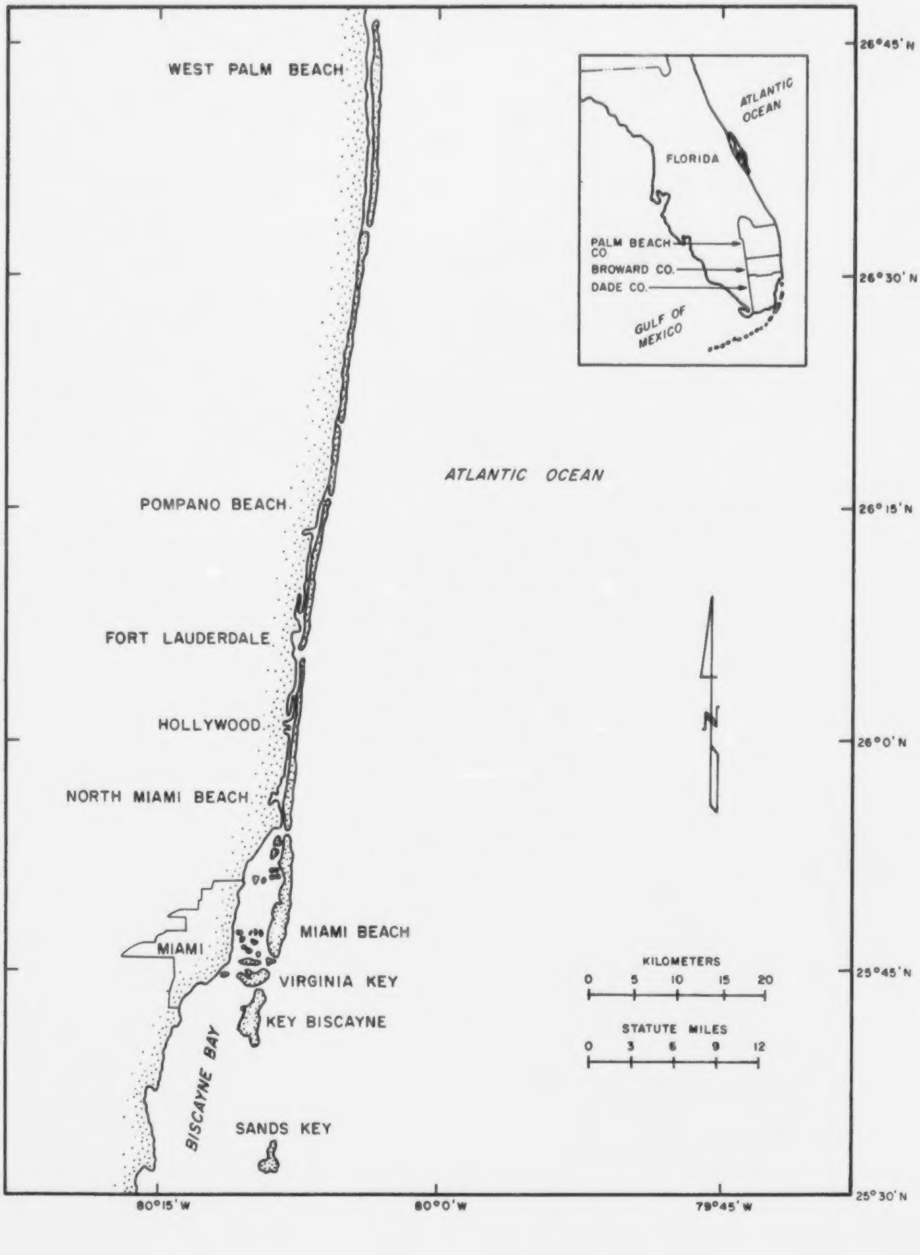
   Dr. Richard Wolke-Histopathologist

   Dr. David R. Young-Environmental Specialist

7. U.S. Environmental Protection Agency
   Dr. Donald J. Baumgartner, Director Marine Division
      - Mr. Robert Brice, Project Officer
      - Ms. Faith Cole, Project Officer
      - Mr. Joseph R. Wanielista
      - Mr. James Westrick

APPENDIX F

STUDY AREA LOCATION MAP



WEST PALM BEACH
POMPANO BEACH
FORT LAUDERDALE
HOLLYWOOD
NORTH MIAMI BEACH
MIAMI
MIAMI BEACH
VIRGINIA KEY
KEY BISCAYNE
BISCAYNE BAY
SANDS KEY
ATLANTIC OCEAN
N
FLORIDA
ATLANTIC OCEAN
PALM BEACH CO.
BROWARD CO.
DADE CO.
GULF OF MEXICO
KILOMETERS
0 5 10 15 20
STATUTE MILES
0 3 6 9 12
26°45' N
26°30' N
26°15' N
26°0' N
25°45' N
25°30' N
80°15' W
80°0' W
79°45' W